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Antibacterial and Antifungal Activity of Flowers of Andrographis paniculata.

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Abstract : Most of the researchers from different parts of the world conducted research mostly focusing on leaves of *Andrographis paniculata* (AP) and few researchers conducted research focusing on stems and roots of the AP. One of the part of AP known as flowers were not concentrated by the researchers with very limited information regarding the AP flower available online. In present research, flower of AP were used to evaluate the antimicrobial properties of the AP's flowers. From the results can be best described that the flowers of AP were having antibacterial and antifungal activities but the strength of zone of inhibition produced can be classified as mild may be due to few reasons. Further evaluation of this flower needed since the phytochemistry was not found online and may be can be concluded that the phytochemistry study has not been conducted yet. The compound/s which lead to antimicrobial activity need to be evaluated.
Keywords: Flower of *Andrographis paniculata*, Chloroform, Methanol, Antimicrobial activity.

Introduction and Experimental

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[1].

Andrographis paniculata or kalmegh is one of the most widely used plants in Ayurvedic formulations[2] and also in Chinese Medicine[3]**.** The Indian pharmacopoeia narrates that *Andrographis paniculata* is a predominant constituent of at least twenty six Ayurvedic formulation[4]**.** *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[5]. It is also used as antibiotic, antiviral, antimicrobial, anti inflammatory, anticancer, anti-HIV, anti-allergic[6]. It is also utilised for common cold, hepatoprotective activity, antimalarial, antidiarrheal and intestinal effect, cardiovascular activity, antifertility activity, pain reduction[7]. 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[8]. It is also having potential to be used as herbicidal and it is used as antiarthritis[1]. In Malaysia, this plant has been extensively used for traditional medicine and help against fever, dysentery, diarrhoea, inflammation, and sore throat[9].

Perhaps different ecological and climatic conditions caused the plant to be introduced as a perennial plant, while most of the references present another botanical definition of the herb as an annual plant. A brittle branched stem, herbaceous plant erecting to a height of 30–110 cm with glabrous, simple, opposite styled leaves and white flowers with rose purple spots on the petals. Even though AP is known as a hermaphroditic, self-compatible and a habitual inbreeding plant, there is an assumed rate of 28% cross pollination for it. Inflorescence pattern extends axillary with terminal panicle or raceme. AP has a fibrous or adventitious root system[1].

This research was conducted to evaluate the antimicrobial activity of flowers of *Andrographis paniculata*. Antimicrobial activity of AP's flower rarely conducted and very limited journals associated with this flower available online with antimicrobial activity of methanol extract of AP's flowers were not found during the literature survey.

Collection of plant materials

The flowers of *Andrographis paniculata* was collected at Sungai Klau, Raub, Pahang, Malaysia within a period time of 6 months .Every time the flowers were collected they were then wash thoroughly under running tap water and dried under shade. They are then finely ground to a powder in an electric blender[10,11]

Preparation of crude extract

The solvents used for the extraction procedure in the present study were chloroform and methanol. About 18 g of dried *Andrographis paniculata*'sflowers powder were weight using electronic balance (AND compony, Japan) and was extracted using 180 ml of the extraction solvents (chloroform and methanol) separately for 48 hours[10,11]. The filtrates was concentrated using a rotavapour (Buchi R-210, Switzerland) at 40°C and then in water bath (Memmert, Germany) until the paste is form[11,12]. The percentage of yield were 3.5 % for chloroform and 3.3 % for methanol. The paste was then kept in air tied container and refrigerated (Sharp, Japan) at 4^{o}C[11].

Bacterial strain

Bacterial strains used in this study were*Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli*. The fungal strain was *Candida albican*and all these strains were obtained from the ASIA Metropolitan University's laboratory.

Antibacterial activity

For the sensitivity testing, the media used were Muller Hinton agar[11,13].4 mg (4000 µg) of extracts (chloroform and methanol) were freshly reconstituted with 50% dimethyl sulphoxide (DMSO) separately (5ml 100% DMSO with 5 ml distilled water). Then they were introduced into two fold serial dilution to obtained 500µg, 250µg, 125µg.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 500 µg / ml, 250 µg / ml, 125 µg / ml[11]. The agar were seeded with 24h culture of the microorganism which met the 0.5 Mac Farland standards. Incubation with incubator (Memmert, Germany) was performed at 37ºC for 24 hours for bacterial strains. Bacterial growth was determined by measuring the diameter of zone of inhibition in millimeters[11,14]. The work was done in triplicate[11,15].

Antifungal activity

Antifungal activity was carried out using the Sabouraud Dextrose Agar(SDA). 4 mg (4000 µg) of extracts (chloroform and methanol) were freshly reconstituted with 50% dimethyl sulphoxide (DMSO) separately (5ml 100% DMSO with 5 ml distilled water). Then they were introduced into two fold serial dilution to obtained 500 µg, 250 µg, 125 µg.Antifungalactivity 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 500 µg / ml, 250 µg / ml, 125 µg / ml[11]. The agar were seeded with microorganism and the plates were incubated at 37ºC for 48 hours[16]. The zone of inhibition were measured using ruler in millimeters.

Statistical analysis : Mean and standard deviation of zone of inhibition were calculated using SPSS version 16.

Table 1: Zone of inhibition of *Andrographispaniculata*'s flower in mm.

	Methanol			Chloroform		
	500 µg	250 µg	125 µg	500 µg	250 µg	125 µg
Staphylococcus aureus	11.67 ± 0.47	8.83 ± 0.24	_	12.50 ± 0.41	9.67 ± 0.47	_
Streptococcus agalactiae	10.00 ± 0.82	_	_	10.67 ± 0.47	_	_
Escherichia coli	10.67 ± 0.47	9.50 ± 0.41	_	12.00 ± 0.82	_	_
Candida albican	8.67 ± 0.24	_	_	_	_	_
DMSO	_	_	_	_	_	_
Mean ± SD						

Table 2: Taxonomy of *Andrographispaniculata*

Kingdom	Plantae, plants
Subkingdom	Tracheobionta, vascular plants
Superdivision	Spermatophyta, seed plants
Division	Angiosperma
Class	Dicotyledonae
Sub-class	Gamopetalae
Series	Bicarpellatae
Order	Personales
Tribe	Justicieae
Family	Acanthaceae
Genus	*Andrographis*
Species	*Paniculata*

Result and discussion

From the table 1, it was clearly stated that flower of *Andrographis paniculata* compose of some mild antibacterial activity against few strains of bacterias. To date most of the research involving AP does not covering the flower part. The researchers mostly focused on the leaves part, with some researchers focused on stem and the roots parts. Very limited information about the flower's antimicrobial activity areavailable in the online access. The reason flower may not be included in the research by most of the researchers because may be it is difficult to be obtained due to its light weight which need to be waited for long time to accumulate the desired amount of the flowers.

Only methanol inhibition zone was recorded for the *Candida albican* with 8.67 mm zone of inhibition. For chloroform, there was no inhibition zone were recorded. The reason is may be the tested concentration is not enough to inhibit the growth of the fungus or the development of the fungal resistance towards the AP's compound which act as antifungal agent.

From the literature survey, got to know that the phytochemistry studies may not have been done for flower extract since no research or review article mentioned about the compound/s present in the flower. Most of the phytochemistry studies was evaluated for the leaves part with this part extracted from the variety of solvents were used for phytochemistry purpose.

The antimicrobial activities of the plant extracted in different solvents varied greatly because there are many factors influence the active compounds present in the plant. The polarity of the extracting solvent are different and greatly influenced the antimicrobial properties[14].

At a concentration of 500 µg and 250 µg, there were inhibition achieved by the both extracts against *Staphylococcus aureus*. Methanol extract produced 11.67mm zone of inhibition while chloroform extract produced 12.50 mm zone of inhibition for the concentration of 500 µg. For the concentration of 250 µg, the zone of inhibition of methanol and chloroform extracts were recorded as 8.83 mm and 9.67 mm respectively. From these finding can be concluded that chloroform extract slightly produce better inhibition than methanol extract. The reason may be the polarity of the solvent or the solubility of compounds better in chloroform than methanol solvent. *Staphylococcus aureus*is one of the gram-positive microorganisms that have been shown to exhibit resistance to a wide range of commonly available antibiotics, especially the penicillins. Therefore, penicillins are often administered in combination with other antibiotics in the treatment of resistant (or suspected resistant) bacterial infections. Most Staphylococci isolated from individuals outside the hospital are resistant to penicillin G due to beta lactamases, which inactivate the drug[17].

At a concentration of 500 µg, the zone of inhibition recorded for the *Streptococcus agalactiae* was 10.00 mm for methanol extract and 10.67 mm for the chloroform extract. Again the chloroform extract showed better inhibition against *Streptococcus agalactiae*. For the concentration of 250 µg, neither methanol nor chloroform produced inhibition.*Streptococcus agalactiae*, recognized in the 1920's as the etiological agent of bovine mastitis[18]. Mastitis is an inflammation of more than 1 lobule of the mammary gland[19]. Group B streptococcus, or*Streptococcus agalactiae*, is a Gram-positive coccus, catalase negative, facultatively anaerobic, spherical or ovoid, and less than 2 µm in diameter; it is usually *β*-haemolytic and is reliably identified by its production of Lancefield group B antigen[20]. In newborns, the most frequent presentations are bacteraemia, pneumonia, or meningitis. In pregnant women *Streptococcus agalactiae* infection causes urinary tract infection, amnionitis, endometritis, and wound infection postpartum. In non-pregnant adultsbacteraemia, genitourinary infection, and pneumonia are the most frequent manifestations. Adults with bacteraemia unrelated to pregnancy are usually elderly and suffer from diseases such as diabetes mellitus, malignancy, liver or renal failure, or AIDS[20].

At a concentration of 500 µg and 250 µg, inhibition zone for *Escherichia coli* by methanol extract were achieved with 10.67 mm and 9.50 mm respectively while for the chloroform extract only at a concentration of 500 µg, the zone of inhibition was achieved. For all of the tested strains at the concentration of 125 µg, no inhibition zone were recorded may be due to insufficient strength of concentration or the strains had developed resistance against the compound/s which responsible for the antimicrobial activities. DMSO which was used as a solvent for freshly reconstitute the plant extract was not producing any inhibition zone since it was used as a negative control in this study.

As a conclusion, flower extract of APof both methanol and chloroform were having antimicrobial activity against few strains but the significance of the study may not met since the zone of inhibition is not big to produce broad spectrum activity. However, the production of zone of inhibition was a prove that this flower possess some antimicrobial activity. In future, the concentration tested need to be increased so that better inhibition can be seen and phytochemistry studies need to be conducted to evaluate which compound or compounds produced antimicrobial activity since the inhibition zone were produced in the present study. Taxonomy of AP was mentioned in the table 2[1].

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