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Synergistic Activity of Chloroform and Methanol Extract of Andrographis paniculata With Erythromycin Against Streptococcus agalactiae

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Abstract: Increasing bacterial resistances are continually recorded in most of the regions in the world. Emergence of this resistance creating major challenges for the healthcare practitioners in order for prescribing the best antibacterial agent for the patients. This research focus on how to reverse such resistance by using medicinal plants.*Andrographis paniculata*(AP), a famous medicinal plant was used in the current research. Research using AP has been extensively done by many researchers but still the synergistic activity of this plant with certain antimicrobial agents has not been conducted yet. From the current study, can be concluded that methanol extract of AP in combination with Erythromycin revealed that it has a bright future as an antibacterial agent to overcome the *Streptococcus agalactiae*bacterial resistance.
Keywords: *Andrographispaniculata*, Chloroform, Methanol, *Streptococcus agalactiae*, Erythromycin.

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]. The taxonomy of AP mentioned in table 1[1].

Streptococcus agalactiae, recognized in the 1920's as the etiological agent of bovine mastitis[10]. Mastitis is an inflammation of more than 1 lobule of the mammary gland[11]. 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[12]. 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[12].

Streptococcus agalactiae is sensitive to many antimicrobial agents, especially β-lactam antibiotics. Among the antimicrobial agents extensively used against *Streptococcus agalactiae* are penicillin G and ampicillin. Penicillin G continues to be the antibiotic of choice for intrapartum prophylaxis for *Streptococcus agalactiae*colonized mothers because of the effective transplacental passage of this agent, its low cost, and broad spectrum of action directed at Gram-positive cocci, with a lower theoretical probability of the emergence of resistant microorganisms[10].

The gastrointestinal tract is the most likely human reservoir of *Streptococcus agalactiae*, with the genitourinary tract the most common site of secondary spread. Colonization rates can differ between ethnic groups, geographic areas, and age groups[12].

The macrolides are a group of closely related antibiotics, mostly produced from *Streptomyces*. The most important therapeutic macrolides are characterized by a 14-, 15- or 16-membered lactone ring. Erythromycin consists of a mixture of compounds in which erythromycin A, which has a 14-member lactone ring, is the active macrolide component. Erythromycin, discovered in 1952, was the first macrolide to be introduced into clinical practice[13].Erythromycin is an acceptable alternatives for mothers who are allergic to penicillin. The prevalence of resistance among invasive *Streptococcus agalactiae* isolates ranged from 7 to 25 per cent for erythromycin[10].

Thus this study was conducted to evaluate the synergistic activity of chloroform and methanol extract of *Andrographis paniculata* with Erythromycin against *Streptococcus agalactiae*.

Collection of plant materials

The leaves of *Andrographis paniculata* was obtained from Sungai Klau, small village in Raub, Pahang, Malaysia. The leaves were then wash thoroughly under running tap water and dried under shade. They are then finely ground to a powder in an electric blender[14,15].

Preparation of agar plates

Blood agar

Beef extract (10.0 g/l), tryptose (10.0 g/l), sodium chloride (5.0 g/l), agar (15.0 g/l)

Suspend 40.0g in 1 litre of distilled water. Heat until completely dissolved. Autoclave at 121^{o}C for 15 minutes. Cool to 50^{o}C. Aseptically add 50 ml defibrinated horse or sheep blood (Liofilchem, Bacteriology product, Italy).

Nutrient broth

Peptic digest of animal tissue (5.0 g/l), beef extract (1.5 g/l), yeast extract (1.5g/l), sodium chloride (5.0 g/l)

Suspend 13.00g in 1000ml of distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired. Sterilize at 15 lbs pressure (121^{o}C) for 15 minutes (Himedia laboratories, India)

Mueller Hinton agar

Beef, infusion from (300 g/l), casein acid hydrolysate (17.50 g/l), agar (17 g/l), starch (1.5 g/l), distilled water (1 liter)

Dissolve 38 grams in 1000ml distilled water. Gently heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121^{o}C) for 15 minutes. Cool to room temperature before use (R & M Chemicals, UK).

Preparation of crude extract

The solvents used for the extraction procedure in the present study were chloroform and methanol. About 25 g of dried *Andrographis paniculata* powder were weight using electronic balance (AND compony, Japan) and was extracted using 250 ml of the extraction solvents (chloroform and methanol) separately for 48 hours[14,15]. 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[15,16]. The percentage of yield was 11.2 % (2.80 g) for chloroform and 11.9 % (2.98 g). The paste was then kept in air tied container and refrigerated (Sharp, Japan) at 4^{o}C[15].

Bacterial strain

Bacterial strain used in this study was *Streptococcus agalactiae*ATCC 13813 which was obtained from the ASIA Metropolitan University's laboratory.

Antibacterial activity

*Streptococcus agalactiae*ATCC 13813 were deposited on the blood agar and incubated for 24 hours at 37^{o}c. Then the bacteria were transferred to nutrient broth and incubated at 37 ^{o}c for 24 hours. For the sensitivity testing, the media used were Muller Hinton agar[15,17].30 mg of extracts (chloroform and methanol) were freshly reconstituted with dimethyl sulphoxide (DMSO) separately. 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 and 200µl of Erythromycin (Oxoid, England) were tested in a concentration of 15µg/ ml separately[15]. 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 strain. Bacterial growth was determined by measuring the diameter of zone of inhibition in millimeters[15,18]. The work was done in triplicate[15,19].

Synergistic activity

The synergistic activity study was calculated by means of cup plate method (Kirby and Bauer technique). Chloroform and methanol plant extract of *Andrographis paniculata* 30 mg/ ml was used in combination with Erythromycin 15 µg/ ml in propotion of 1:1 (100 µl : 100 µl). 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[15].

Table 1: 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*

Table 2: Zone of inhibition in (mm) of individual and combination activity of AP extracts with erythromycin against *Streptococcus agalactiae*ATCC 13813

	Chloroform	Methanol	Erythromycin	Chloroform + Erythromycin	Methanol + Erythromycin
*Streptococcus agalactiae*ATCC 13813	23.33 ± 0.47	23.67 ± 0.47	27.00 ± 0.00	31.33 ± 0.47	32.67 ± 0.47
DMSO	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Mean ± Standard Deviation (SD)

Results and Discussion

The zone of inhibition was measured by using the ruler. From the table 2, clearly stated that chloroform and methanol extract of *Andrographis paniculata* were having antibacterial properties against *Streptococcus agalactiae*ATCC 13813. The leaves part were used in the present research. From the previous studies mentioned that andrographolide from *Andrographis paniculata*'s plant was playing vital role in antimicrobial properties of the plant[3]. The taste of andrographis is very bitter. This bitterness is related with its various pharmacological properties[6].

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[18].

The inhibition zone of chloroform extract AP yielded 23.33 mm of zone of inhibition against *Streptococcus agalactiae* ATCC 13813 and the methanol extract of AP yielded 23.67 mm. These 2 findings proved that methanol extract of AP was slightly better than chloroform extract. This may due to the polarity of the solvents used.

According to a group of researchers, they had mentioned that they used 6 mm diameter of well for antimicrobial activities and the zone of inhibition of 8 to 10 mm were considered significant[20]. According to the statement above, the present study met it's significant of the zone of inhibition since the well size was 8mm and the inhibition zone greater than 23 mm which produced broad spectrum activities.

The zone of inhibition achieved by erythromycin was 27.00 mm. This inhibition was greater than chloroform and methanol extract of AP. 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. This type of action by erythromycin causes the bacteria to be killed due to the protein synthesis inhibition within the bacteria[15].

Combination of erythromycin with chloroform extract of AP and erythromycin with methanol extract of AP lead to synergistic effects with the zone of inhibition were 31.33 mm and 32.67 mm respectively. To date research on synergism is very limited[21]. The mechanism of action of erythromycin was mentioned above but what lead to the synergistic activities need to further analysed. As mentioned above, the andrographolide played important role in inhibiting the growth of *Streptococcus agalactiae*. These double attack of erythromycin (protein synthesis inhibition) and andrographolide (need to do further evaluation about the target site/s) may lead to the synergism. Comparing two sets of data of synergism, combination of erythromycin and methanol produced better synergism than combination of erythromycin and chloroform extract. So, can be concluded that the methanol solvent is better to be used for extracting the compounds from *Andrographis paniculata*. Again the reason may be the different polarity of the solvent were used and the degree of solubility of the bioactive compounds in the respective solvents.

In the present study, as mentioned above, although the preferred drug of choice for treating *Streptococcus agalactiae* is penicillin but in the condition of patients allergic to penicillin, erythromycin can be prescribed. The reason erythromycin was chosen for the synergistic study because since the bacterial resistance are emerging now, the resistance of *Streptococcus agalactiae* towards erythromycin were recorded previously. So, for that reason to identify any possibilities of AP in combination with erythromycin being used as an alternative choice by the medical practitioner to reverse such resistance was carried out.

Specifically in macrolides, three mechanisms of resistance have been described, comprising preventing the drug from reaching its target where limiting the access of macrolides to the cells (efflux pumps), altering the target where altering the ribosome to prevent effective binding of macrolides and inactivating the antibiotic in which bacterial production of inactivating enzymes[15,22].

Conclusion

Methanol extract of *Andrographis paniculata* in combination with erythromycin can be used as a alternative choice to reverse the bacterial resistance of *Streptococcus agalactiae* towards the erythromycin. Further evaluation needed to find out the bioactive compound which lead to the synergism in the present research.

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References

1. Alireza V., Mihdzar A.K., Soon G.T.,Daryush T., Mohd P.A. and Sonia N., Nain-e Havandi *Andrographis paniculata* present yesterday, absent today: a plenary review on underutilized herb of Iran's pharmaceutical plants. *Int J Mol CellBio.*, 2011,39(5), 5409-5424.
2. Vijaykumar K., Murthy P.B.S., Kannabab S., Syamasundar B. and Subbaraju G.V., Estimation of Adrographolide in Andrographis paniculata Herb, Extracts and Dosage forms, *Int J ApplSci Eng.*, 2007, 5(1), 27-39.
3. Chowdhury A., Biswas S.K., Raihan S.Z., Das J. and Paul S., Pharmacological Potentials of Andrographis paniculata: An Overview. Int J Pharm., 2012, 8, 6- 9.
4. Panneerselvam C., Ponarulselvam S. and Murugan K., Potential Anti-plasmodial Activity of Synthesized Silver Nanoparticle using *Andrographis paniculata*Nees (Acanthaceae). *Arc ApplSci Res., 2011*, 3(6), 208-217.

5. Patidar S., Gontia A.S., Upadhyay A. and Nayak P.S., Biochemical Constituents in Kalmegh (*Andrographis paniculata* Nees.) Under Various Row Spacing's and Nitrogen Levels.*World ApplSci J.*, 2011, 15(8), 1095-1099.
6. Jegathambigai R., Devaraj S., Kumar P. and Sivaramakrishnan S., Study on the hepatoprotective effect of Andrographis paniculata (Burm. F) nees on mice. Journal of Phytology., 2010, 2(11), 25- 30.
7. JarukamjornK. and Nemoto N.,Pharmacological aspect of *Andrographis paniculata*on health and its major diterpenoid constituent andrographolide. *J Health Sci., 2008,* 54(4), 370-381.
8. Akbar S., Andrographispaniculata: A Review of Pharmacological Activities and Clinical Effects. *Alt Med Rev*., 2011, 16(1), 66-77.
9. Kirar K., Kaurav D., Chourasiya J. and Shukla RN., (2012). Extraction and identification of diterpenoid lactone from andrographis paniculata,Int j pharmacol res dev., 2012,4(10),53 – 56.
10. Beitune P.E., Duarte G. and Maffei C.M.L., Colonization by Streptococcus agalactiae During Pregnancy: Maternal and Perinatal Prognosis, The Brazilian Journal of Infectious Diseases., 2005, 9(3), 276-282.
11. Arroyo R., Martín V., Maldonado A., Jimenez E.,Fernandez L. and Rodriguez J.M., Treatment of Infectious Mastitis during Lactation: Antibiotics versus Oral Administration of Lactobacilli Isolated from Breast Milk, Clin Infect Dis, 2010,50(12), 1551-1558.
12. Scanziani R., Dozio B., Baragetti I., Grillo P., Colombo L., Liso S.D. and Surian M., Vaginal colonization with group B Streptococcus (Streptococcus agalactiae) and peritonitis in a woman on CAPD, Nephrology Dialysis Transplantation., 1999,14(9), 2222-2224.
13. Hawkyard C.V. and Koerner R.J., The use of Erythromycin as a gastrointestinal prokinetic agent in adult critical care: benefits versus risks, J Antimicrobial Chem., 2007, 59, 347–358.
14. Soma R., Kiranmayee R., Bhuvaneswari C.H., Archana G. and Lakshmi N.M,.Phytochemical analysis of *Andrographis paniculata* extract and its antimicrobial activity, World J. Microbiol.Biotech., 2009, 26(1), 85-91.
15. Sivananthan M. and Elamaran M., In vitro evaluation of antibacterial activity of chloroform extract Andrographis paniculata leaves and roots, Duriozibethinus wood bark and Psidiumguajava leaves against selected bacterial strains, Int J Biomol Biomed., 2013, 3(1), 12- 19.
16. Rusmiati., The effects of durian wood skin methanol extract (DuriozibethinusMurr) of Ovarium and Uterus Microanatomy Structure Female Mice (Musmusculus L), SainsdanTerapan Kimia., 2010, 4(2), 108- 118.
17. Al-Haddad A.M. Urinary tract infection among pregnant women in Al-Mukalla district, Yemen, EastMediterr Health J., 2005, 11(3), 505-510.
18. Kataky A. andHandique P.J., Antibiotic Activity and phytochemical estimation of micropropagated Andrographis paniculata (Burm.f) Nees. Asian JSciTech., 2010, 5, 91- 94.
19. Alagesaboopathi C. andKalaiselvi N., Antimicrobial activities of the root, stem and leaf extracts of Argemonemexicana L,Int J Bio., 2012, 2(5), 61- 68.
20. Hema T.A., Arya A.S., Suseelan B., Celestinal J.R.K. and Divya P.V., Antimicrobial activity of five south indian medicinal plants against clinical pathogens, International Journal of Pharma and Bio Sciences., 2013, 4(1), 70-80.
21. Kumar A.S., Venkateshwaran K., Vanitha J., Saravanan V.S., Ganesh M., Vasudevan M. andSivakumar T., Synergistic activity of methanolic extract of Thespesiapopulnea (Malvaceae) flowers with oxytetracycline, Bangladesh J Pharmacol, 2009, 4, 13-16.
22. Soares G.M.S., Figueiredo L.C., Faveri M., Cortelli S.C., Duarte P.M. andFeres M., Mechanisms of action of systemic antibiotics used in periodontal treatment and mechanisms of bacterial resistance to these drugs, J Appl Oral Sci., 2012, 20(3), 295- 309.

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