A STASTISTCAL APPORACH IN DETERMINING THE CHEMICAL AND BIOLOGICAL STUDY OF ANDROGAPHIS PANICULATA

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ABSTRACT

Chemical investigation of the dichloromethane extracts of Andrographis paniculata Nees led to the isolation of andrographolide (1), 14-deoxyandrographolide (2), 14-deoxy-12-hydroxyandrographolide (3), a mixture of β-sitosterol (4a) and stigmasterol (4b) in a 3:1 ratio, and chlorophyll a (5) from the leaves; a mixture of 4a and 4b in a 3:2 ratio, 5,2'-dihydroxy-7,8-dimethoxyflavone or skullcapflavone I (6), and a mixture of long chain trans-cinnamate esters (7a) and β-sitosteryl fatty acid esters (7b) from the roots; 4a, monogalactosyl diacylglycerols (8), lupeol (9), and triacylglycerols (10) from the pods; and 2 from the stems. The structures of 1-3 and 6 were elucidated by extensive 1D and 2D NMR spectroscopy, while the structures of 4, 5 and 7-10 were identified by comparison of their NMR data with those reported in the literature.

The dichloromethane extracts of the leaves of Andrographis paniculata led to the isolation of neoandrographolide (1), 1,5-dimethyl-1,5-cyclooctadiene (2) and 2-hydroxyethyl benzoate (3), and squalene (4) from the leaves, while the stems yielded 1 and 2. The structure of 1 was elucidated by extensive 1D and 2D NMR spectroscopy.

1.0 INTRODUCTION

Medicinal plant Andrographis paniculata belong to the family 'Acanthaceae' which nick name-Kalmegh, Maha tita and Kalpanath. It is an annual herb that can grow from 30-100 cm tall. The stem is distinctly four angular. Leaves are opposite, simple and narrowly egg-shaped to lance shaped. Flowers are in lax, auxiliary and terminal racemes or panicles combined into a pyramidal inflorescence. A. paniculata is distributed in tropical region of Asia, India, Hong Kong, Thialand, Brunel, Singapore and Bengaladesh. It can be found in a variety of habitats, such as plains, hill sides, coastlines and cultivated area such as roadsides, farms and waste lands.

The climatic requirement for the plant is hot and humid conditions with ample sunshine. Depending upon area of cultivation harvesting is done in October to November. The flowering and fruiting is done throughout the year especially from August to May. Andrographis paniculata has long been used in Indian system of medicine "Ayurveda". Medicinal properties of plants had been mentioned in pharmacopoeias of India, Korea and China. Local herbal healers use A. paniculata to prevent and cure from fever, intestinal worm and infections.

Herbal medicines have been main source of traditional medicines. The active principle compounds of plant activate metabolism through liver due to which many necrotic debris remove rapidly and cure mostly physiologically disorder in human body. The plant possess febrifuge, tonic alternative, stomachic, anthelmintic and cholagogue properties and also used in different types of liver complaints, colic constipation, cholera, dysentery, diarrhoea, diabetes, dyspepsia, general debility, hook work infection, hyper acidity, influenza, bronchitis, malaria, swellings and itches, piles, gonorrhea, scabies, stomach disorder and stomachache. It is also used as a cure for torpid liver and jaundice.

In many part of India it is used as curative or preventives in snake venom poisoning. It is chiefly used is viral hepatitis, diminished appetite and drug induced liver damage. All above properties of A. paniculata are prescribed by Ayurvedic physician for clinical treatment. A decoction of the plant is used as a blood purifier and a cure for torpid. Leaves contain two bitter substances lactones named andrographolide and kalmeghin. The ash contains sodium chloride and potassium salts. Plant is very rich in chlorophyte. The plant have diterpenoids, and rographolide, 14-deoxy-11-oxo-andrographolide, 14-deoxy-11,12- didehydroandro grapholide, 14-deoxy and rographalids and neoandrographolide.

The roots gives flavones apeigenin-7, 4'-dio-O-methyl ether, 5-hydroxy-7, 8, 2´, 3´-tetra methoxy flavone, andrographin and panicolin and α-sitosterol. Leaves of kalmegh contain homoandrographolide, andrographosterol and andrographone. Active compounds of Kalmegh sold in market by different trade names Acene-n-pimple cream (Himalaya wellness), Liv 52 (Himalaya Drugs Company), Lerbohep (Lupin Herbal Laboratory), Sage Liverex (Sage Herbals), Purodil syrup (Aimil Pharmaceuticals). Most of Vaidya suggest decogition for different types of physiological disorder in human beings. Villagers used this plant leaves for first aid treatment of different insect bite and infections.

Medicinal value of Andrographis paniculata

The medicinal value of these plants is due to some chemical active substances that produce a definite physiological action on the human body. Many medicinal plants and herbs are active against antimicrobial activity. Methanol extracts of Andrographis paniculata have antimicrobial activity against two bacterial pathogen viz., Pseudomonas aeruginosa and S. aureus. Andrographis paniculata found a valuable medicinal plant in many popular systems of medicine including Ayurveda, Siddha and Unani. It cures dysentery, antivenom, fever, cholera, diabetes, influenza and swelling. The decoction of this plant used against jaundice. The extract of this plant exhibit antifertility, anti-fungal and antinematicidal activities.Andrograpis paniculata Snake repellent and locally called or in Tamil Nilavembu, Siriyanangai and Periyanangai in Tamil Nadu.

Headaches, nasal and throat symptoms and general malaise showed the most improvement. Ayurveda and other traditional medicinal system for the treatment of diabetes, describe a number of plants used as herbal drugs. It has a lower side-effect and reasonable cost. The active principles present in medicinal plants have been reported to possess the pancreatic beta cells re- generating, insulin

releasing and fighting the problem of insulin resistance . Andrographis paniculata contains diterpens, locations and flavonoid, flavonoids mainly exist in the root, but also can be isolated from the leaves. The leaves contain two bitter lactone andrographolide, and kalmegh, Active compounds extracted with ethanol or methanol from the whole plant, leaf and stem of Andrographis paniculata include over 20 diterpenoids and over 10 flavonoid.

2.0 LITERATURE REVIEW

Fincher, G.T., W.G. Monson and G.W. Burton, (1981) The taxonomy of the genus Bolboceras Kirby, 1819 (= Indobolbus Nikol ajev, 1979) is discussed. The Oriental members of the genus as currently conceived have not been revised for the past 160 years. Operational groups of species are proposed, including the Bolboceras nigricans group.

Anderson, J. M. and M. J. Coe. (1974) The knowledge on Atlantic Forest scarab beetle fauna is quite limited. This biome is strongly degraded and these insects can be used as bioindicators since they are sensitive to forest destruction and show distinct organizational patterns in forest fragments or in areas that have been deteriorated by human activity. The lower winter richness was most pronounced in the conserved valley, while richness remained relatively constant in the degraded valley; abundance was much higher in the degraded valley. The cluster analysis showed that the valleys and hillsides are the most similar in relation to species composition and abundance, yet different from the secondary forest with eucalypts and the hilltop.

Kakkar, N. and S.K. Gupta, (2009) Hunting often impacts rain forest mammal communities but little is known about its indirect effects on other taxa. We examined dung beetle assemblages using pitfall and flight-intercept traps at six rain forest sites in Panama that ranged in hunting intensity. **Hingston, R.W.G., (1923)** Dung beetles are important components of most terrestrial ecosystems. In tropical rain forests, dung beetle communities can be very rich in number of species and individuals, and they are known to be useful bioindicators of habitat disturbance. Dung beetles captured during the dry season were only found in the SDF. When comparing the beginning and the middle of the rainy season, differences in abundance and guild structure were also observed between both periods and between forest types, but these differences were much less pronounced.

Halffter, G. and M.E. Favila, (1993) Invertebrate focal taxa that can act as information surrogates for broader patterns of biodiversity are of increasing interest to conservation practitioners. Scarabaeine dung beetles have been widely proposed as an ideal group for biodiversity inventory and monitoring, they satisfy all of the criteria of an ideal focal taxon, and they have already been used in ecological research and biodiversity survey and conservation work in many regions of the world. Here I review the characteristics that make the Scarabaeinae suitable for this purpose and suggest future directions for the broader use of this group as a focal taxon.

Fincher, G.T., T.B. Stewart and R. Davis, (1970) The aim of this study was to compare the dung beetle (Coleoptera, Scarabaeidae: Scarabaeinae) community structure at two sites in the Charles Darwin Ecological Refuge in Igarassu, Pernambuco, Brazil. Dung beetles were collected in

2006 using monthly samples from 48 pitfall traps baited with human dung and bovine carrion.

Haffter, G. and E.G. Matthews, (1966) Species diversity and abundance of scarabaeoid dung beetles (Coleoptera) attracted to fresh cow dung were studied in three habitats of New Jersey: Hutcheson Memorial Forest (HMF) disturbed field, HMF old growth forest, and Rutgers University Bovine Farm.

3.0 METHODOLOGY

Materials Alloxan and glibenclamide were purchased from Sigma Chemical Co. (St.Louis, MO, USA). Glucose level were measured using colorimetric method (GOD-PAP) with glucose oxidase and 4-aminoantipyrine (DiaSys, Diagnostic Systems GmbH, Holzheim, Germany). Sodium carboxymethyl cellulose and glucose were obtained from E. Merck, Darmstadt, Germany. All other reagents were high-grade qualified materials. Animals Wistar rats (2-3 month old) weighing 150-200 g used in the study were maintained on a constant temperature (22 ± 2 °C) and a constant relative humidity (55 ± 10%) and automatically controlled 12:12 h light-dark cycle (light on at 07:00 a.m.).

They were fed with a standard laboratory food and water at libitum. Ethical clearance for the animal study was obtained from Research Ethics Committee, Integrated Research and Testing Laboratory Universitas Gadjah Mada, Indonesia. Preparation of ethanolic extract A. paniculata (Burm. f.) Ness and A. indica A. Juss were collected from Sleman, Yogyakarta during October 2013. Plant authentication was performed at Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia. The voucher specimen was stored in a herbarium of the department.

The dried leaves were powdered and then stored in an airtight container for further use. The plant was dried and powdered separately, extracted by maseration using 70% (v/v) ethanol for 24 hours with ratio of 1:10. After twice re-extraction, collected filtrate was evaporated under reduced pressure to get a viscous extract. The extracts of A. paniculata and A. indica A. Juss were processed into dried extracts, then mixed with ratio by 1:1. Phytochemical analysis Analysis of ethanolic extract of A. paniculata was performed using a stationary phase of silica gel 60 F254 and a mobile phase of chloroform-ethyl acetate-methanol-acetic acid (7:2:0,5:0,5 v/v).

Detection of andrographolide content in spot was performed under UV wavelength 254 nm. Standard andrographolide and Andrographis paniculata Ness extract were prepared in methanol solvent at concentration of 1 mg/ml and 4 mg/ml, respectively. Quantitative analysis was performed by measuring spot intensity in TLC scanner at wavelength of 230 nm. In the phytochemical analysis of A. indica ethanolic extratct, TLC method using stationary phase of silica gel 60 F254 and a mobile phase of n-butanol-acetic acid-water (4:1:5 v/v) was used.

Rutin was applied as a marker compound and spot detection was performed under UV 254 and UV 366 nm. Quantitative analysis of rutin content in the extract was performed using spot scanning at wavelength of 364 nm.

Induction of hyperglycemia

The rats were induced by an intraperitoneal injection of alloxan monohydrate to achieve

hyperglycemic condition at single dose of 150 mg/kgBW. Control group rats were treated with saline solution. Hyperglycemic condition was determined at 72 hours after induction by measuring preprandial and postprandial blood glucose levels. Rats with blood glucose level >216 mg/dL (>12 mmol/L) were categorized as diabetic condition and compared to blood glucose level of normal control group.

Collection of plant material

The fresh leaves of the plant Andrographis Paniculata were collected during the month of October 2015 from the area of Trichy. The fresh leaves of that plant were taxonomically identified from the Rabinet Herbarium (RHT) of St. Joseph College, Trichy. Extraction of plant material Healthy plant leaves were collected, washed thoroughly in tap water and dried in room temperature for 30 days. The dried leaves were powdered and 25 g leaf powder soaked separately in 225 ml of ethanol for 3 days. The extracts were filtered through whatman No.1 filter paper. Similar process was repeated twice with fresh solvent and the filtrate was collected together. The extract was stored at the refrigerator for further studies.

Screening of phytochemical components

The leaf extracts of A.Paniculata were analysed for the presence of alkaloids, carbohydrates, saponin, protein, phytosterol, phenolic compounds, flavonoid and glycoside according to the common phytochemical methods described by Harborne (1998). Microorganisms Pure culture of Escherichia coli, Staphylococcus aureus and Bacillus subtilis specie of bacteria and Candida albicans, Aspergillus niger and Aspergillus flavus specie of fungi were procured from, the Department of Microbiology of Thanjavur Medical College, Thanjavur.

4.0 RESULTS

Phytochemical analysis of A. paniculata

Based on phytochemical analysis using TLCdensitometry method, ethanolic extract of A. paniculata showed positive content of andrographolide (Fig. 1). Qualitative detection under UV 254 exhibited a same spot with standard andrographolide (hRf 53). Furthermore, quantitative analysis confirmed the content of andrographolide by 16.17% using TLC scanner at wavelength of 230 nm.

Phytochemical analysis of A. indica

Phytochemical analysis of ethanolic extract of A. indica was performed using rutin. The content of standard compound was determined based on rutin concentration in A. indica extract (Fig. 2). Qualitative detection was observed under UV 254 and UV 366 and showed the presence of rutin in A. indica extract. Further analysis using AlCl3 spray exhibited higher intensity of rutin spot that was detected under UV 254 and UV 366. Quantitative analysis of rutin concentrationin extract using TLC scanner at wavelength of 364 nm resulted in rutin level of 2.86%.

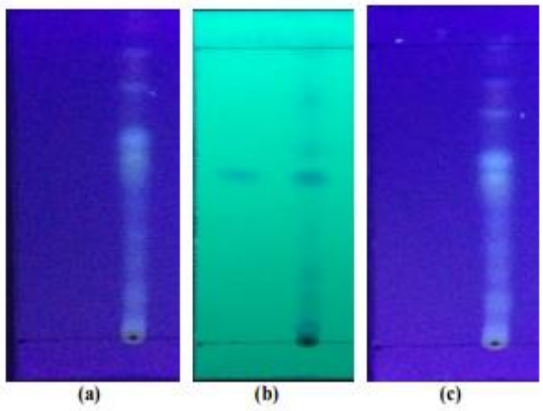


Figure TLC profile of ethanolic extract Andrographis paniculata (Burm. f.) Ness. TLC

method was performed using a stationary phase of silica gel 60 F254 and a mobile phase of chloroform-ethyl acetate-methanol-acetic acid (7:2:0,5:0,5 v/v). Spot detection (a) under UV 366nm, (b) under UV 254 after AlCl3 spraying, and (c) under UV 366 after AlCl3 spraying

Effect on blood glucose levels

Both preprandial and postprandial blood glucose levels raised significantly after single dose administration of alloxan (150 mg/kgBW) intraperitoneally in rats. Determination of blood glucose level was performed at 72 hours after alloxan induction. Figure 3 shows that alloxan-induced group had higher blood glucose level in comparison to normal control group.

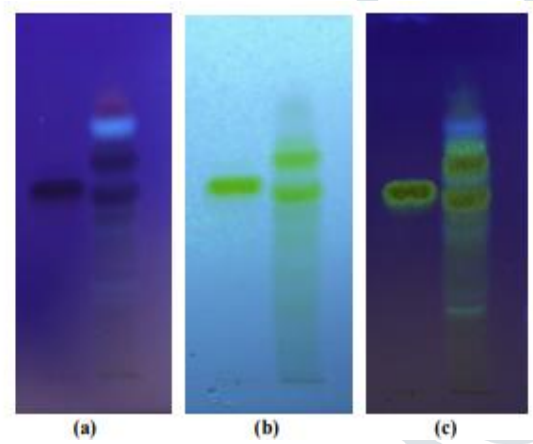
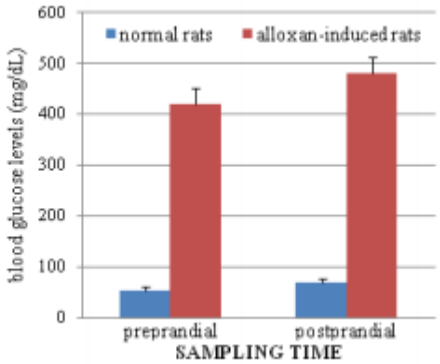


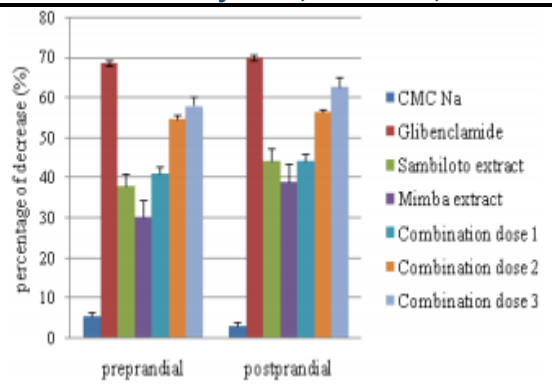
Figure TLC profile of ethanolic extract Azadirachta indica A. Juss. TLC method was performed using a stationary phase of silica gel 60 F254 and a mobile phase of n-butanol-acetic acid-water (4:1:5 v/v). Spot detection (a) under UV 366nm, (b) under UV 254 after AlCl3 spraying, and (c) under UV 366 after AlCl3 spraying.

As a control, there was no significant change in blood glucoce level after treatment CMC-Na (fig 4). As shown in table 1-2 and figure 4, administration of combination of A. paniculata and A. indica for 15 days reduced preprandial and postprandial blood glucose levels in alloxan-induced diabetic rats. In comparison to single ethanolic extract of A. paniculata or A. indica, the combination exhibited higher activity to reduce blood glucose level. Treatment of diabetic rats with the combination at dose of 200 mg/kgBB demonstrated more reduced blood glucose level in comparison to single extract treatment.



Graph Profile of preprandial and postprandial blood glucose levels in rats after alloxan induction in comparison to normal control group. Data represent mean±SEM, and are five to six independent experiments.

The reduction of blood glucose level was enhanced as increased dose of combination treatment as shown in group VI (combination 400 mg/kgBW) and group VII (combination 800 mg/kgBW). At the highest combination dose, blood glucose level preprandial and postprandial was reduced by 58.121% and 62.839%, respectively. However, the effect of blood glucose reduction at highest combination administration was still lower than this of standard diabetic drug, glibenclamide, that reduced preprandial blood glucose by 68.782% and postprandial blood glucose by 70.25%.



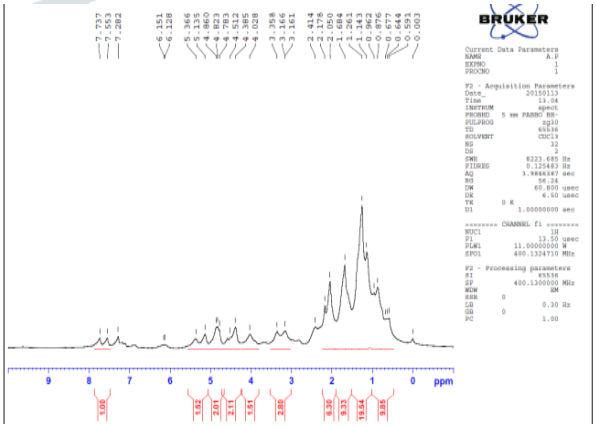
Graph Hypoglycemic activities (%) of all treatments. Dose 1-3 are 200, 400 and 800 mg/kg BW, respectively. Data represent mean±SEM, and are four to five independent experiments.

Phytochemical screening of the ethanolic extracts of A.Paniculata revealed the presence of alkaloids, carbohydrates, saponin, protein, phytosterol, phenolic compounds, flavonoid and glycoside. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases.

For example, Alkaloids protect against chronic disease. Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show the analgesic properties. The Steroids and saponins were responsible for central nervous system activities ,flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities.

Andrographis paniculata consist several therapeutically important active principle compounds in the aerial parts. [18, 19, 20]. AGPs were further characterized by FTIR for denoting the presence of the different functional groups. The evolution of this studies FT-IR absorbance of characteristic band structures was followed by establishing the ratios between the main observed peaks. The compounds show the leading bands in the regions of 1250- 1020 cm-1, 3000- 2850 cm-1, 1680- 1640 cm-1, 1550- 1475 cm-1 and 1550- 1475 cm-1. The appearance of these peaks reveals that the presence of various functional groups like aliphatic amines, alkanes, alkenes, nitro compounds aromatics, alcohols and phenolic groups.

The peak value of 31.94 indicates the 14-deoxy-12-hydroxy andrographolide-19- O-β-Dglucuronide. The chemical shift peak value of 61.67 expose the glucose, 27.23 and the 29.71 showed neo androrapholide (C26H4308). Following the peak value was 32.20 and 29.71 indicate the neoandrographolide the peak value of 135.97 showed the α, β- unsaturated γ- lactone ring was present, then the following the peak value 39.35, 27.23 was shown neoandrographolide and 167.71 was shown a methoxy group. 13C NMR spectrum six characteristic aromatic carbons at δ 166.1, 165.2, 161.0, 98.5, 96.1 and 95.4 indicated the occurrence of an unsymmetrically substituted phloroglucinol unit.



Graph H NMR chemical shifts of flavonoids in methanol

The therapeutic properties include 14- deoxy-11-oxoandrographolide, 14- deoxy -11-12-

didehydroandrographolide D, 14- deoxy-andrographolide, non-biter compound neo andrographolide, homo andrographolide, andrographosterol, andrographane, andrographosterine, andrograpanin, α-sitosterol, stidmasterol, apigenin- 7, 4-di-O-methyl ester, 5-hydroxyl 7,8,2,3- tetramethoxy flavones, monohydroxy trimethyl flavones, andrographnin, dihydroxy-di- methoxy flavones, panicolin, andrographoneo, andrographoside, andropaniculosin, isoandrographapholide and skull caflavone.

5.0 CONCLUSION

Live attenuated vaccines elicit both humoral and cellular immunity similar to that elicited by the natural infection, but they may pose some risk such as reversion to pathogenicity within an immunized individual. On the other hand, inactivated vaccines may be safer than live vaccines, but their antigens elicit only antibody responses and essentially no CMI. The new type of DNA (or nucleic acid) vaccines are composed of a bacterial plasmid encoding foreign antigens cloned in eukaryotic expression vectors. This new type of vaccines is being developed because of the potential to induce both the humoral and the cellular arms of the immune response.

If one could tailor the immune response towards CMI, the potential to develop safe inactivated vaccines capable of generating CMI that provide protection against intracellular infectious diseases would become possible. Traditional herbal medicines are widely used for the treatment of many kinds of acute and chronic diseases in Asia. Some herbs, such as Echinacea, Astragalus, Picrorrhiza, Phytolacca and Andrographis has been claimed to be immune enhancing herbs. However, herbal extracts are a mixture of bioactive and inactive chemical ingredients. Also, the chemical composition of extracts varies depending on geographical distribution, climate condition of cultivation and the method used for preparation of the extracts. Hence, it is desirable to validate these bioactive chemical ingredients responsible for the claimed therapeutic properties.

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