Biological screening of selected traditional medicinal plants species utilized by local people of Manokwari, West Papua Province

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Abstract. Lense O. 2011, Biological screening of selected modificitual medicitual plants species atilized by local people of Manohvari, West Papua Province, Nucamura Bioscience 3: 145-150. The aim of the research was to determine the presence of alkaloids and antimicrobial activity in extracts from selected medicitual plants from Manokwari District, West Papua, Indonesia. The method of alkaloid testing followed the standard phytochemical methods. The procedure of the Calibrated Dichotomous Sensitivity (CDS) test was used for the antimicrobial bioasnays. Results of biological screening suggested that all but one of the 56 species tested contained different levels of alkaloids. Eleven species showed anti-microbial activity using bioasnays of responses to two bacturia, Salworella typhi and Klebsiella pneurosiae, and two fangi Candide albicaus, and Cryptococcus moformane; none of the plant estracts showed an antimicrobial effect against the bacteria. Escherichia coli. Extract of Plancoordia sp. was the most active one as it showed activity against three different organisms (C. albicau, C. neoformanu, and S. right).

Keywords: biological screening, local people, Manokwari, traditional medicinal plant, West Papua,

Abstrak. Lense O. 2011. Penapisan hayati beberapa jenis tambulaan obat wadisional serpilih yang dimanfamkan oleh masyarakat lokal Manokwari, Provinsi Papua Barat, Nasantara Biomismee 3: 145-150. Tajuan penalitian ini adalah untuk mengetahai adanya alkaloid dan aktivitas anti-mikroba ekstesk beberapa tanaman obat tenpilih dari Kabupaten Manokwari, Papua Barat, Indonesia. Metode pengujan alkaloid mengikati metode fitokimia standar. Prosedur uji Colthrated Dichotomous Sessititety (CDS) digunakan untuk uji tayati anti-mikroba. Hasil penapisan hayati menunjukkan bahwa ke-56 jenis yang diuji mengandung alkaloid dengan kadar yang berbeda-beda, kacuali satu jenis. Sebelas jenis menunjukkan aktivitas anti-mikroba berdusarkan respons uji hayati terhadap dua bakteri. Sabnosella typhi dan Kleinista presenoniae, dan dua jamir Conside albicans dan Cryptocuccur neoformous, tidak sategun dai eksteak tunaman yang menunjukkan albivas ke-tadap tiga organisme yang berbeda (C. albicans, C. menforman), dan S. raphi).

Kata kunri: penapisan biologi, masyarakat lokal, Manokwari, tambuhan obat tradisional, Papan Barat-

INTRODUCTION

Tropical rainforests with their high levels of diversity are considered to have great potential as a source of new drugs. The global trend of going "natural" or "green" has also contributed to the tropical rain forest is a target for such activities, combined with the added fear of forest depletion caused by logging. transmigration, and other developmental activities. Screening for biological activity using simple and fast bioassays is now being used to identify potentially useful plants. Phytochemical separations are routinely guided by bioassays which will ensure the isolation of bioactive agents inrespective of whether they belong to a certain class of compound or not.

The Manokwari tropical miniforest comprises a very rich and characteristic flora that covers more than 30,000 square kilometers of West Papua. Many of the plants in the forests have been used as traditional medicines by the local people living in the area in order to treat several tropical diseases including malaria, fever, dysentery, wounds, and fungal or hacterial infections (MacKinnon 1991). However, no phytochemical analyses of medicinal plants from the Manokwari region have been conducted.

Fungi and bacteria cause important human diseases in tropical regions, especially in immunocompromised or immunodeficient patients. Despite the existence of potent antibiotic and antifungal agents, however, resistant or multi-resistant disease strains are continuously appearing, imposing the need for continuous research for and development of new drugs (Silver and Bostian 1993). In an effort to discover new compounds, many research groups have screened plant extracts to detect secondary metabolites with relevant biological activities.

The aim of the present study was to determine the presence of alkaloids and anti-microbial activities in extracts from selected medicinal plants from Manokwari District, West Papua, Indonesia.

MATERIALS AND METHODS

Collecting the samples

Samples of potentially useful plants were collected in the field from February to April 2000 in collaboration with the State University of Papua (UNIPA), Manokwari, West Papua Province, Indonesia. Specimens were collected at the same time for identification. Samples for laboratory analysis were chosen from the plants that are used as medicine sources by traditional healers (Martin 1995). Plant parts such as leaves, fruits, flowers, bark, stems, and roots were collected for biological screening.

Preparing and preserving the samples

Samples of fresh plant parts such as leaves, fruits, flowers, hark, stems, and roots were broken or cut into suitable sizes for transport. Plant parts such as roots and bark were chopped into pieces using clippers. All plants were air-dried before being transported to the laboratory, where they were dried in an oven at a maximum temperature of 50°C for 72 hours or more depending on the water content of the samples (Martin 1995).

Analysis of the samples

Alkaloid screening

The method of alkaloid testing followed the procedures of Culvenor and Fitzgerald (1963); Frelich and Marten (1973). Seven and half gram of finely ground plant material was rapidly extracted with 75 mL of ammoniacal chloroform (CHCh). After filtration, the solution was extracted by adding 9 mL of sulphuric acid. Three milliliters of extract was then transferred to a test tube and 9 drops of silicotungstic acid added (12 g silicoturgstic acid to 100 mL water). The presence of alkaloids in the extract phase was detected by the formation of a precipitate. Where the results were positive, the amount of alkaloid present was visually assessed and ranked into five classes according to the relative abundance of the precipitate (Collins et al. 1990; Barr et al. 1993).

Anti-microbial screening

The procedure of calibrated dichotomous sensitivity test (Bell et al. 1999) was used for the anti-microbial bioassays. In the laboratory, 2.5 g of dry finely ground plant material was grounded into a powder and then divided into samples for different mixed with 50% and 90% ethanol, and shaken for 24 hours. The extracts were filtered and left to stand for 24 hours under vacuum at 40°C. Under sterile conditions, 5 µL of extract was applied to a disc of filter paper and placed on an agar plate that had been inoculated with a single species of bacterium (Salmonella typh), Klebstella pneumoniae, and Escherichia coil) or fungus (Camikia albicons, Cryptococcus neoformans), all of which are human pathogens.



Figure 1. The activity of curracts of *Litsea* sp. and *Planchowella* sp. against *Candida albicans*. The filter paper discs represent the plant extracts that were extracted using 50% and 90% EIOH. The clear zone indicated the plant extract was effective against *C. albicans*.

After inoculation, inverted plates were incubated for 18-24 hours at 35°C. Inhibition of growth of the bacteria and fungi by the plant extracts was examined by measuring the diameter of the clear zone (a microbe-free circle) that might form around the impregnated filter paper disc. If the disc showed clear zones of 7 mm or more, the microbes were considered vulnerable to inhibition by the plant extract and that the plant displayed anti-microbial activity. In contrast, if the clear zone was 6 mm or less, it indicated that the microbes were resistant to the plant extract (Martin 1995). Figure 1 shows an example of agar plate used in anti-microbial activity screening. It shows that the extract of *Planchonella* sp. was effective against *C. albicans*, whereas the extracts of *Litnea* sp. showed no activity against *C. albicans*.

RESULTS AND DISCUSSION

Alkaloid screening

Fifty-eight ethanolic extracts of various parts of 56 plants used as traditional medicinal plants were investigated for the presence or absence of alkaloids. All but one of these (55 species; 98%) contained various levels of alkaloids (Table 1), but only six appeared to have a high level of alkaloid presence (Figure 2).

The results show a much higher percentage of plants giving a positive alkaloid response than similar studies elsewhere. For example, a survey conducted on endemic species in Tasmania, Australia, indicated only15% of the species have a positive alkaloid reading (Bick et al. 1996). In a study on alkaloids of medicinal plants from Lombok,

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Plant species	Family	Localities	Medical conditions	Parts tested (results)
Acorus calamus L.	Araceae	Ratsiki, Anggi	Dysentery	Rhizomen (++++
Adenanthers microgreenes	Manosaceae	Manokwari	Epilepsy, diamhea, queasy, fever	Bark (++++)
Ageratum convigoides	Asterioceae	Wasior, Minyambouw	Wound	Leaves (++++)
Alpinia purpurata	Zingiberaceae	Kebar, Ransiki	Earaches	Stem(+++)
Alstonia scholaris R.Br.	Аросупасеае	Ratsiki, Kebar, Wasior, Manokwari	Fever, Malaria	Bark (+++++)
Artocarpus conunants	Moracesie	Ransiki, Anggi, Kehar, Wasiot, Mendey	Wounds, genorthen	Bark (++++)
Biophytum petersianum	Oxalidaceae	Kebar	Desire of having a child	Leaves i++++i
Bhonea sonañks	Asteraceae	Ransiki, Angei	Cold, influenza	Leaves (++++)
Calophyllaus inophyllaus L.	Guttifenae	Ransiki	Initiated eyes	Leaves (++++)
Canavius sp.	Biorstructure	Ramaiki	Liver diseases	Bark (++++)
Casevariea rumphiana	Cassarinaceae	Manokwari	Malaria	Bark (++++)
Coclogue aperata	Orchidaceae	Mendey	Chest pain	Bulb (+++)
Coforasia sp.	Araceae	Ransiki, Angei	Chrildbirth	Bulb (+++)
Connetine nudifloru	Commelinaceae	Ransiki, Anggi	Dysentery	Leaves (+++)
Condyüne fraticosa	Liliacean	Runsiki, Anggi,	Dysentery, irritated eyes	Leaves (+++)
Costus speciosus (Koen) Sw.	Zingiberaceae	Minyamboaw Merdey	Ear pain, stomachaches, food	Stem (+++)
Diplazium cocalemum (Retz.) Sw	Polypodiaceae	Kebar	poisoning Headaches, wounds	Leaves (++)
Disordon arborenous Miq.	Meliacene	Kehar	Malaria and strong favor	Bark (++++)
Drymaria quercificita 1 S m	Polypodiaceae	Minyambouw	Feyer, malaria	Leaves (+++)
Dryopaevii sp.	Polypodiaceae	Wasior, Kebar	Snakebine	Leaves (+++)
Endospermum moluccomum	Euphorbiaceae	Ransiki	Fever	Bark (+++)
Evonio sp.	Rataceae	Mendey	Ashma	Bark (++++)
Firas sp.	Moraceae	Ransiki, Anggi, Kebar	Asthma	Bark (++++),
Ficus sp2.	Moraceae	Wasior	Abscess, chest pain	Twigs (+++) Leaves (+++).
Giganuschlen sp.	Poseene	Wasior	Toothaches	Roots (+++) Outer back
	# 312 CONT		A Print and a second second	[++++)
Gueruu guenen	Gnetaceae	Merdey	New wounds	Back(++++)
Horoslannes autons (Forst £)	Euphorbiaceae	Ratsiki, Anggi, Wasior,	Liver diseases	Loaves (++++)
Guillemin		Kebar		
Hoesfieldia sp.	Myrislicacene	Mendey	Stomachaches	Birk (+++)
Intsis palembanica	Caesalpiniaceae	Merdey	Stomachaches	Bark (++)
Lansiani domenticaro Jack,	Meliaceae	Wasion	Dyseniery	Bark (+++)
Laporte a interrupta (L.) Chew,	Urticaceae	Kobar	Maloria	Leaves (+++)
Litocorpus brasii	Fagaceae	Kebar	Muscular pain	Bark (++++)
Litaca sp.	Lauraceae	Manokwari, Minyambearw	Scabies	Back (++++)
Lorannes sp.	Loranthaceae	Merdey	Gotornhoea	Leaves (++++)
Macaranga tanarina	Euphorbiaceae	Ransiki, Anggi,Kebar	Fever (babies)	Leaves (++++)
Mucana novoguinensis	Fabaceae	Ransiki, Kebar	Diarthoea, malaria, fever	Leaves (+++)
Nauclea orientalis	Rubiaceae	Minyambouw, Merdey	Easy birth	Shoot (++++)
Octomeles annationa Mig.	Distincent	Ransiki, Anggi	Fever	Bark (++++)
Palandon sp.	Sapotaceae	Merdey	Unspecified men sexual diseases	Bark (++++)
Pentophalangian pachycarpum A.C. Smith.	Clusiaceae	Ransiki, Anggi	Hinge pain	Bark (+++)
Pineleodendoon ambomicum HSK	Euphorbiaceae	Ransiki, Anggi, Kebar, Merdey	Headaches, unspecified men sexual diseases	Leaves (+++)
Piper sp.	Pipemorae	Wastor, Ransiki, Anggi	Stomachaches	Leaves (+++)
P(puaras repondas (Bl), Wedd,	Unicaceae	Ransiki, Anggi, Mordey, Manokwari	Fever, dianthea, epilepsy	Bark (+++)
Pisento sp.	Nyctaginacene	Merdey	Headaches	Roots (+++)
Planchensella sp.	Sapotaceae	Mercley	Dysenlery	Bark (++++)
Polygonian sp.	Polygonaceae	Wastor, Kebar	Scabies	Root (++++)
Polygonau sp.	Polygonaceae	Kebar	Dysentery	Leaves (++++)
Potlass scandens	Araceiae	Mendey	Diarrhea	Leaves (+)
Prevocarpus indicas Willd. Rhaphidophora oblorgifisha	Papilionaceae Anaceae	Kebar Wasior	Dysentery New wounds	Bark (++++) Leaves (++++)
Scott. Rhaphidophore permise Roxb.	Алжене	Wasior, Merdey	Liver diseases, unspecified men	Leaves (+++)
Ricinus constantis L.	Euphorbiaceae	Ratsiki	sexual diseases Malaria, decoction before	Leaves (++++)
Schiamatogloris cal-aptra Roab.	Anaceaet	Kebar	delivering a haby Dislocated knee or arms	Leaves (+++)
Scindap.nus hederocens	Amoene	Merdey	Colds of infants	Leaves (+++)
Spathodza companulati	Bignoniaceae	Minyambouw	Tonic	Bark (++++)
Anathog lortis sp.	Orchidaceae	Mercley	Wounds	Bulbs (+++)

Table 1. Manikwari medicinal plants species giving negative and positive test results for alkaloids.

 Spankogloritis sp.
 Orchidaceae
 Merdey
 Woulds
 Data (++++)

 Note: The symbol in the bracket in the last column indicate the level of alkaloids presented: (-) no alkaloid, (+) very low, (++) low, (+++) medium, (++++) medium-high, and (+++++) high level of alkaloids presented.
 In alkaloid, (+) very low, (++) low, (+++)

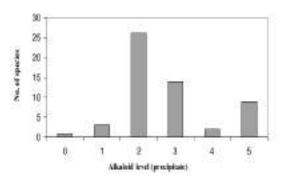


Figure 2. Frequency distribution of the qualitative amount of alkaloids in 56 species medicinal plants from Manok wari District giving positive tests for alkaloids (5 is high).

23% of the medicinal plants tested showed positive result for alkaloids (Hadi and Bremmer 2001). In a similar alkaloid survey from Queensland, Australia, involving many tropical and sub-tropical species, 20 % of the species tested gave positive result (Hadi and Bremmer 2001). In a phytochemical survey of medicinal plants in Sayap-Kinabalu Park, Sabah, Malaysia, where 60 species were tested for alkaloids, only eight species (13.3%) gave positive results (Said et al. 1998).

Some of the species tested for alkaloids have been reported to contain alkaloids and other active compounds. The thizomes of Acorus columns contain leucoantho-cyanins and 5,7-dihydroxyflavanol (Cambie and Brewis 1997). The active ingredient in A. columns is b-asarone which belongs to the phenylpropanoid family (Baxter et al. 1960). The species of A. columns contained the greatest amount of basarone (70-96%) (Streloke et al. 1989), including engenol, methyl-eugenol, acorin, calamenol, calamene, calameone (Woodley 1991); cincole, linalcol, pinene, resins, safrole, and tamains are also reported (Cowan 1999).

Hadi and Bremner (2001) reported that the leaves, bark, and roots of Alstonia scholaris and Ficus septicy contain unknown alkaloids. The seeds of these species are rich in hallucinogenic indole-alkaloids (alstovenine, venenatine, chlorogenine, reserpine, ditamine, echitamine) and chlorogenic acid (a mild bladder and urethra irritant, resulting in increased sensitivity of the genital region), whereas the only alkaloids present in the bark and lates are ditamine, echitamine, and echitenine.

Ming (1999) reported that Agevataw cowyzoidez contains alkaloids, mainly the pyrrolizidinic group, which suggest that it may be a good candidate for pharmacological studies. Alkaloid has been found in the species, with hepatotoxic activity including 1,2-desifropyrolizidine and licopsamine. Alkaloids also were found in a hexane extract of A. cowyzoidez in Africa (Wiedenfeld and Roder 1991). Menut et al. (1993) reported that this species contained high percentage of precocene 1, particularly those plants from Nigeria and Cameroon which were rich in precocene 1, while oil extracted from Vietnamese and Fijian (Sova) plants contained roughly the same amounts of both compounds, Terpenoids, steroids, Flavonols, glucosides and polyoxygenated flavones have been isolated from plants from India, China, Nigeria, and Notthern Vietnam. Monoterpene a-princine and eugenol have been detected in Indian plants, and o-farnesene, humulene and caryophyllene oxide have been identified in Fijian plants (Menut et al. 1993). Hormones agenatochromene and 7methoxy-2, 2-methylchromene (precocene-1) form 60 % of the total essential oils from the flowers, leaves, and stems of a Fijian variety (Aalbersberg and Singh 1991).

The seeds of Lansium domenticum are known to contain an amount of an unnamed alkaloid, 1% of an alcohol-schuble resin (Morion 1987), and triterpenes (Bunyapraphatsara and Saralamp 1982). Bunyapraphatsara and Saralamp (1982) found only anti-inflammatocy activity confined to the fractions containing triterpenes in seed extracts. The nonpolar triterpene fraction showed systemic activity in a rat carrageenin-induced model of inflammation while the polar fractions reduced ear inflammation. The findings confirmed the efficacy of the seeds of *L. domesticum* in reducing ear inflammation (Saralamp and Bunyapraphatsara 1995).

Cowin (1990) reported that the seeds of *Ricinus* communis contained up to 3 % of the toxalbumin ricin. This is one of the most toxic substances known. They also contained alkaloid ricinine, cyanogenic glycosides, flavonoids, steroidal sapogenin, garlic acid, and potassium nitrate, and the oil is rich in ricinoleic, stearic, undecylenic acid, and ricinine (Grainge and Ahmed 1988), 19

Moreover, some other genera documented in this study have been reported to contain alkaloids and other compounds. The thizomes of Alpinia galanga (L.) Willd, reported containing kaempferia, galanga, a volatile oil, and galanged (which yields cincole), pinene, and eugenol (Perry 1980). The extract of stem and leaves of Blannea holscariferia (L.) DC, contain alkaloids and tannins flavonoids (Grainge and Ahmed 1988; Bhuiyan et al. 2009). Fruits of Piper galaneense Schum, & Thonn, contain the amides piperine, N-iso-butyloctadeca-trans-2-trans-4-dienamide, sylvatine, m-,β-dihydropiperine and trichostachine, and P. nigram has pipercide, dihydropipercide, and guineensine (Miyakado et al. 1989). The essential oil from the berries is composed of the terpenes: phellandretic, pinene, and limotiene (Oliver 1986).

Said et al. (1998) reported that the leaves of Lithocorpus confragoous contained saponin (3+); the leaves and the bark of Litrea elliptibacea contained alkaloid (2+) and saponin (2+); the leaves of Ficus hemsleyana, F, lepicurpa, F, rubrocupidata, and F, stolonifera contained saponin (2+, 2+, 3+, and 3+ respectively), and Palaquium sp. (leaves) contained saponin (3+).

Anti-microbial activity screening

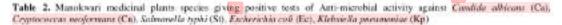
Of the 56 plant extracts tested in an agar diffusion assay, 11 species were effective against the two gramnegative bacteria (*Klebstella pneumonlae*, and *S. typhi*) and two fungi (*C. albicans*, *C. neoformans*) assayed.

Planchonella sp. was the most active species, showing activity against 3 different organisms (C. albleans, C. neoformans, and S. typhi; Table 2 and Figure 3) followed by Adenanthera microsperma and Dysoxylum arborescens,

both of which were effective in two bioassays (C. neoformani and Klebsiella pneumoniae). C. neoformani was the most susceptible of the two yeasts tested, with 7 extracts from a total of 11 extracts displaying activity against this organism. Against C. neoformanis, the extracts from Ficus sp2, showed very significant inhibition (22.75 mm inhibition zone), followed by Dysoxylum arborescens (20.25 mm inhibition zone) and Laportea interrupta (17.50 mm inhibition zone). On the other hand, the extracts from Alpinia purpurata and Lithocarpus brassii showed less significant inhibition (7.5 mm inhibition zones) against C. neoformanis and C. albicans respectively. None of the plant extracts was effective against Exclorible zon.

The results of the laboratory-based anti-microbial activity screenings of plant species from Manokwari District suggested why some traditional medicinal plants might be

effective against certain medical conditions. The bark of the stem of Planchonella sp. Adenanthera microsperma, and the leaves of Loranthus sp. are very commonly used by the native people in Manokwari District to treat dysentery, diarrhea, and fever. The plant extracts of these species were effective against S. rypli which is one of the pathogenic microbes causing fever, diarrhea, and headaches (Wasfy et al. 2000). The use of the bark of stems of Lithocarpus brassii in treating ringworm has also been supported by the anti-microbial screening results. The extracts of this species were confirmed effective against C. albicans which is an opportunistic organism (yeast) causing an itchy rash and occurs most often in warm, moist areas, such as under the arms, between skin folds, and in the groin (Bartie et al. 2001). Candida also causes mouth infections, particularly in babies and elderly.



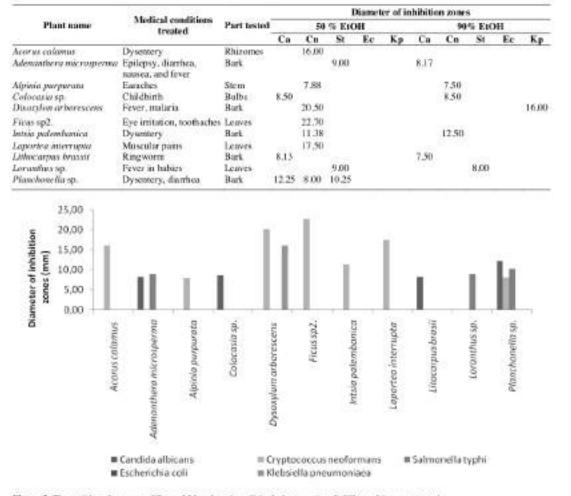


Figure 3. The activity of extracts of Several Manokwari medicinal plants against 5 different bioassays tested.

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In addition, the anti-microbial screening indicated that the extracts of fresh leaves of the nettle Laportea interrupto and the bark of the stem of Dysoxylum arborescens were very effective against C. neoformans that can cause fatigue and fever (symptoms of pneumonia; Kopecka et al. 2000). This finding agrees with the use of Laportea interrupta and Dysoxylum arborescens in this region to treat muscular pains for fatigue and fever, respectively (Table 2). However, there is no previous information regarding preparations of antibiotics from Laportea sp. to treat this pathogen, although Foster and Duke (1990) reported that it has shown antibacterial and central nervous system depressant activity.

CONCLUSION

Initial work on Manokwari medicinal plants has resulted in fifty-six species being collected and screened for the presence of alkaloids and anti-microbial activity. Results indicated that at least 55 species of the 56 species ninforest species analyzed were shown to contain different level of alkaloids. Anti-microbial activity tests indicated that 11 species were effective against three Gram-negative (Escherichia coli, Klebsiella pneumoniae, and Salmonella typhl) bacterial species and two fungi (Candida albicanx, Cryptococcus aceformans). Planconella sp. was the most active species as it showed activity against three different organisms (C. albicans, C. neoformans, and S. typhi).

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