8	Java Chili	*Piper retrofractum*	Piperaceae	Fruit
9	Cloves	*Syzygium aromaticum*	Myrtaceae	Flower
10	Ciplukan	*Pysalis angulata*	Solanaceae	Leaf, Fruit
11	Pomegranate	*Punica granatum*	Lytraceae	Leaf, Fruit
12	Insulin	*Smallanthus sonchifolius*	Asteraceae	Leaf
13	Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome
14	Guava	*Psidium guajava*	Myrtaceae	Leaves
15	Distance	*Jatropha curcas*	Euphorbiaceae	Leaf
16	Lime	*Citrus aurantiifolia*	Rutaceae	Fruit
17	Kaffir Lime	*Citrus hystrix*	Rutaceae	Leaves
18	Cumin	*Nigella sativa*	Ranunculaceae	Seeds
19	Katuk	*Sauropus androgynus*	Euphorbiaceae	Leaves
20	Cinnamon	*Cinnamomum burmannii*	Lauraceae	Stem
21	Kedawung	*Parkia timoriana*	Fabaceae	Seeds
22	Coconut	*Cocos nucifera*	Arecaceae	Fruit
23	Moringa	*Moringa oleifera*	Moringaceae	Leaves
24	Basil	*Ocimum sanctum*	Lamiaceae	Leaves
25	Kemuning	*Murraya paniculata*	Rutaceae	Leaves
26	Kencur	*Kaempferia galanga*	Zingiberaceae	Rhizome
27	Cat's Whiskers	*Orthosiphon aristatus*	Lamiaceae	Leaves
28	Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome
29	White Turmeric	*Curcuma zedoaria*	Zingiberaceae	Rhizome
30	Lavender	*Lavandula angustifolia*	Lamiaceae	Flower
31	Lawang	*Illicium verum*	Illiciceae	Flower
32	Lempuyang	*Zingiber zerumbet*	Zingiberaceae	Rhizome
33	Galangal	*Alpinia galanga*	Zingiberaceae	Rhizome
34	Aloe Vera	*Aloe vera*	Asphodelaceae	Leaves
35	Mangosteen	*Garcinia mangostana*	Clusiaceae	Skin
36	Noni	*Morinda citrifolia*	Rubiaceae	Fruit
37	Nutmeg	*Myristica fragrans*	Myristicaceae	Seeds
38	Papaya	*Carica papaya*	Caricaceae	Leaf, Fruit
39	Salam	*Syzygium polyanthum*	Myrtaceae	Leaves
40	Sambiloto	*Andrographis paniculata*	Acanthaceae	Leaf
41	Secang	*Caesalpinia sappan*	Fabaceae	Stem
42	Senggani	*Melastoma candidum*	Melastomataceae	Leaves
43	Lemongrass	*Cymbopogon citratus*	Poaceae	Stem, Leaf
44	Betel Green	*Piper betle*	Piperaceae	Leaves
45	Red Betel	*Piper ornatum*	Piperaceae	Leaves
46	Soursop	*Annona muricata*	Annonaceae	Leaves
47	Srikaya	*Annona squamosa*	Annonaceae	Leaves
48	Suket Teki	*Cyperus rotundus*	Cyperaceae	Tuber
49	Telang	*Clitoria ternatea*	Fabaceae	Flower
50	Temuireng	*Curcuma aeruginosa*	Zingiberaceae	Rhizome
51	Temukunci	*Boesenbergia rotunda*	Zingiberaceae	Rhizome
52	Temulawak	*Curcuma Zanthorrhiza*	Zingiberaceae	Rhizome

Table 1 describes the medicinal plants often used by the Sedayu Village community. Ginger (Zingiber officinale), a rhizome plant belonging to the Zingiberaceae family, had the greatest percentage of medicinal plant utilization at 88.5% (Fig. 1). Ginger rhizome has many well-known health benefits, including anti-inflammatory and antioxidant properties, as well as the ability to relieve nausea. In addition, ginger is also used to treat digestive problems (Linda & Lovadi, 2013). Besides ginger, the plant with the highest medicinal plant use was kencur (Kaempferia galangal) at 84.6%. People in Indonesia frequently use kencur to treat vomiting, vertigo, cough, and other digestive issues. The greatest percentage of medicinal plant use was also found in turmeric (Curcuma longa), at 73.1%. In medicine, turmeric can be used as a pain reliever for women who are menstruating and increase appetite to treat liver disease (Arum et al., 2012)

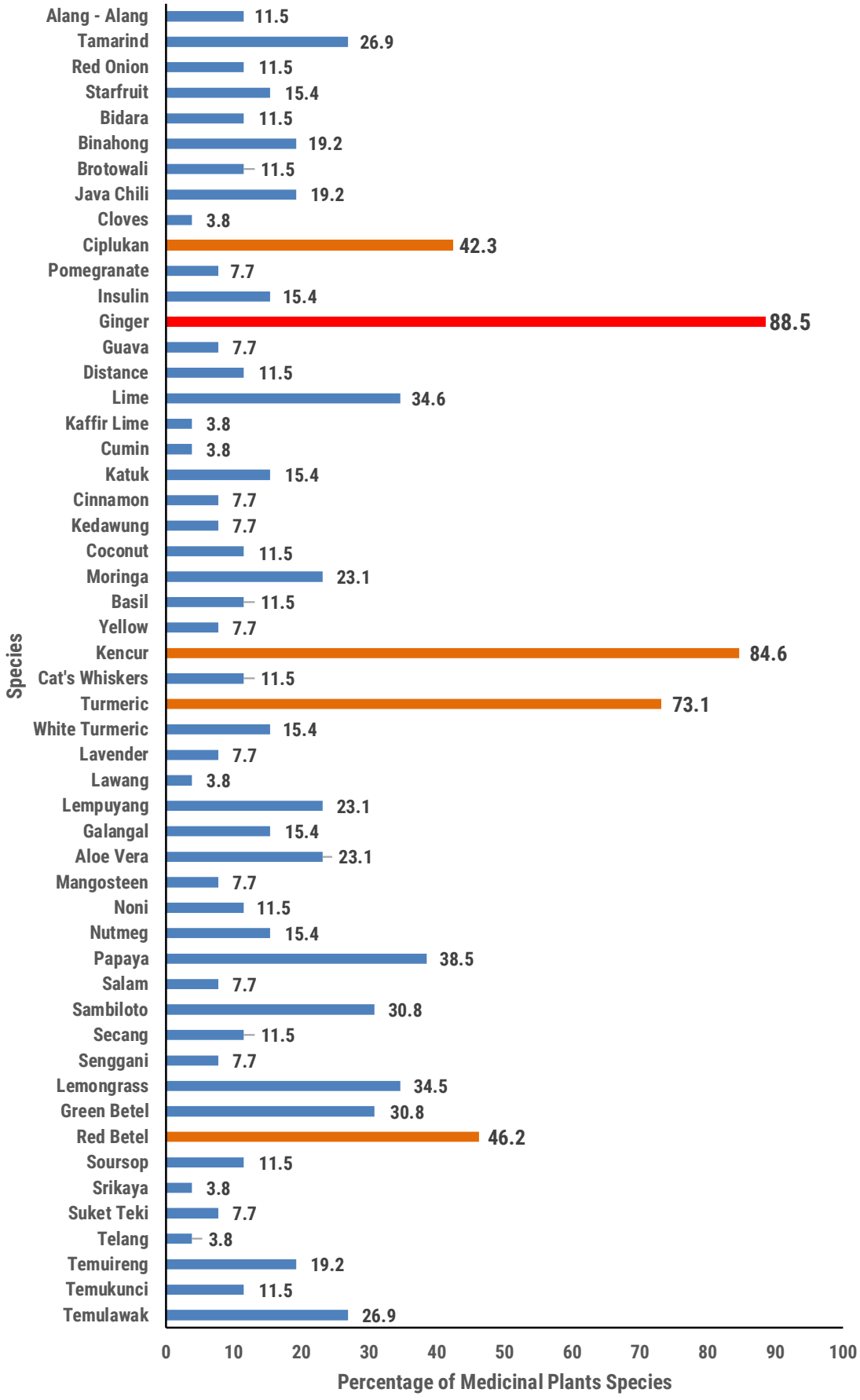


Figure 1. Percentage of medicinal plant types used by the Sedayu village community

Plant parts (organs) used by the Sedayu Village community as medicine were roots, stems, seeds, fruits, flowers, leaves, skin, rhizomes, and tubers. The leaves of 22 varieties of medicinal plants (42.6%) were the most commonly used part (organ) of medicinal plants. Figure 2 depicts the proportion of plant parts that were used as ingredients in medicine.

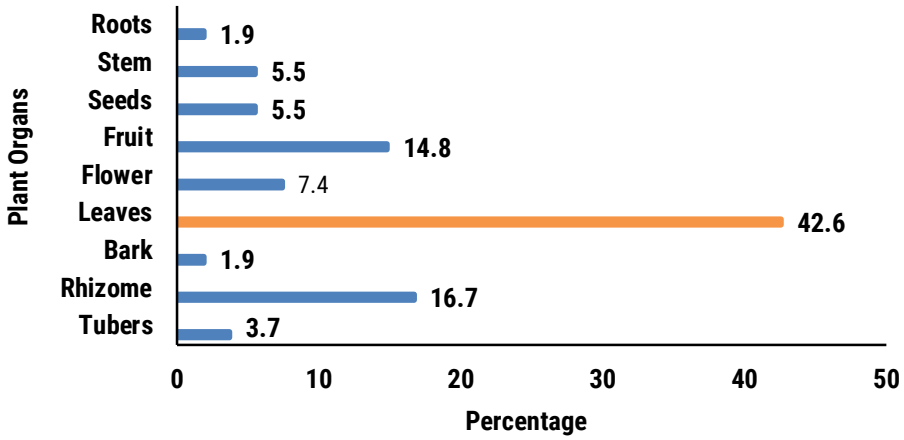


Figure 2. Percentage of Plant Organs Utilized as Medicine

Leaves are more widely used because they are considered easy to obtain and process and have greater efficacy than other plant parts. Furthermore, the most convenient component to extract or gather from the sources is the leaves (Farhatul, 2013). In addition, leaves have a high water content (80%) and contain essential oils, phenols, potassium compounds, and chlorophylls that are capable of curing diseases (Nulfitriani et al., 2013). Similar research results were also found in the Maybrat Tribe community in Sembaro Village, Ayam Maru district, South Sorong Regency, where the leaves are the most widely used part as medicinal plants (Howay et al., 2003). The Dayak Iban community predominantly utilizes leaves from plants for medicinal purposes (Meliki et al., 2013).

The use of other plant parts, such as stems, rhizomes, tubers and roots, are more difficult to collect because they require uprooting and cutting for their utilization, which ecologically affects their amount in nature (Fadilah et al., 2015). This is in accordance with the results of research by Febrianti and Krisnawati (2021), namely, the selection of types and ingredients from medicinal plant parts can be efficacious to reduce or cure certain diseases optimally through processing stages derived from roots, stems, and leaves with the right dosage.

Based on the results of research from 26 respondents with 52 species of medicinal plants, processing can be done in one of five ways: boiled then consumed, brewed then consumed, mashed then applied, squeezed, fermented, or unprocessed. Some varieties of medicinal plants can be processed in multiple ways, such as kencur, which can be consumed directly (without processing), mashed, then applied, or boiled and consumed. If the disease or complaint was distinct, the processing was performed differently. Figure 3 shows the percentage of medicinal plant utilization based on the processing method.

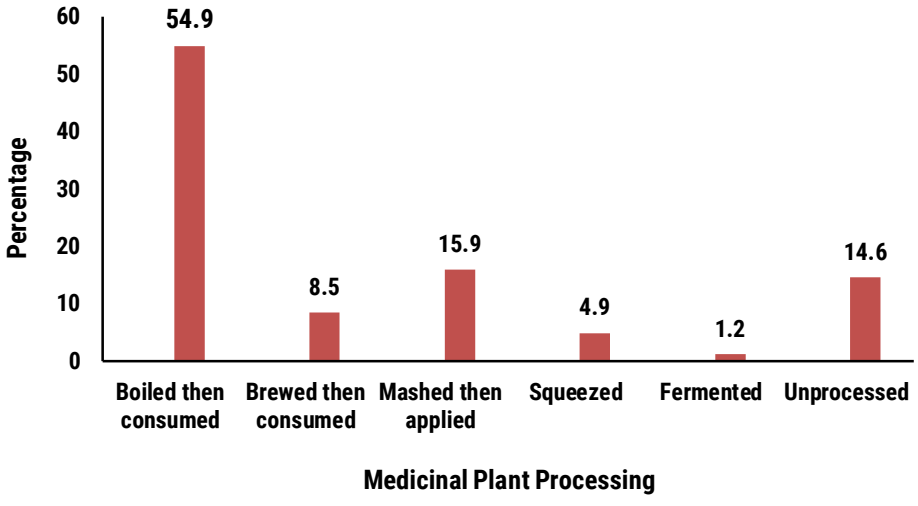


Figure 3. Percentage of medicinal plant processing methods

Figure 3 shows that medicinal plants based on the processing method are mostly boiled and then consumed (54.9%). Similar research results were also found in the Baluran National Park community, where boiling and then consuming plants is the most widely used method (Yaqin, 2020). The Osing Tribe community also shows that boiling and then consuming is the most widely used way of utilizing medicinal plants (Utomo, 2017). The community believes that applying the boiling procedure to medicinal plants eliminates any pathogens present in the plants. Boiling is also believed to be effective because, generally, the diseases treated are types of internal diseases. Another possibility related to the processing method, people who do it more by boiling is because it is easier to take the juice or properties of the plant (Wulandara et al., 2018). The medicinal plant parts used are boiled first and then consumed as an internal disease treatment. Additionally, locals hold the belief that ingesting medicinal plants for internal ailments produces a more rapid effect and reaction than utilizing them topically and even more rapidly than employing other methods to utilize medicinal plants (Efremila et al., 2015).

The advantages of treatment using medicinal plants are that they do not cause side effects compared to using modern drugs or drugs from chemicals. Also, certain herbs are easily available around the yard and easy to make. The process of making traditional medicines is generally very simple, including those that are brewed with water, made into powder and then dissolved in water; some are taken with juice, and the treatment method is generally done orally (drunk) (Azmin et al., 2019). Medicinal plants that are processed in the form of concoctions are generally in the form of jamu, which is formulated with medicinal plants as the basic ingredient and added with other supporting ingredients found in nature (Arisandi & Andriani, 2011).

Due to the benefits of traditional medicine, conservation is one of the means by which medicinal plants must be preserved. Furthermore, knowledge pertaining to the refining and application of medicinal plants must be transmitted orally and through direct practice in order to ensure its eternal sustainability. As awareness of the advantages of medicinal plants grows, it is anticipated that the community will become more engaged in endeavours to preserve these plants, thereby ensuring the continued generation of knowledge as local wisdom.

CONCLUSION

The Sedayu Village community utilizes a total of 52 different species of medicinal plants, with ginger being the most commonly utilized (88.5%). The Sedayu obtain medicinal plants through cultivation, the wild, or the market; leaves are the most utilized plant organs (42.6%). Processing and utilization of medicinal plants by the Sedayu community is mostly done by boiling and then drinking (54.9%). In order to preserve knowledge regarding medicinal plants, the Sedayu community engages in sustainability actions, such as imparting this information to children and local residents through direct or oral practice.

REFERENCES

Arisandi, Y., and Andriani, Y. (2011). *Khasiat Berbagai Tanaman untuk Pengobatan Berisi 158 Jenis Tanaman Obat [Benefits of Various Plants for Medicine Contains 158 Types of Medicinal Plants]*. Jakarta: Eska Media.

Arum, G.P.F., Retnoningsih, A., and Irsadi, A. (2012). Etnobotani Tumbuhan Obat di Desa Keseneng, Kecamatan Sumowono, Kabupaten Semarang [Ethnobotany of Medicinal Plants in Keseneng Village, Sumowono District, Semarang Regency]. Unnes Journal of Life Science, 1(2): 126-132.

Azmin N., Rahmawati A., and Hidayatullah, M.E. (2019). Uji kandungan fitokimia dan etnobotani tumbuhan obat tradisional berbasis pengetahuan lokal di Kecamatan Lambitu, Kabupaten Bima [Test the phytochemical and ethnobotanical content of traditional medicinal plants based on local knowledge in Lambitu District, Bima Regency]. *Florea: J Biol Pembelajarannya,* 6: 101- 113.

Dharmono, D. (2018). Kajian Etnobotani Tumbuhan Jalukap *(Centella Asiatica L.)* Di Suku Dayak Bukit Desa Haratai 1 Loksado [Ethnobotanical Study of Jalukap Plant *(Centella Asiatica L.)* In Dayak Hill Tribe of Haratai Village 1 Loksado]. BIOSCIENTIAE, 4(2).

Efremila, Evy, W., and Lolyta, S. (2015). Studi etnobotani tumbuhan obat oleh etnis suku Dayak di desa Kayu Tanam kecamatan Mandor kabupaten Landak [Ethnobotanical study of medicinal plants by Dayak ethnic tribe in Kayu Tanam village, Mandor sub-district, Landak regency]. Jurnal Hutan Lestari, 3(2), 234-246.

Fadilah, Lovadi, I., and Linda, R. (2015). Pemanfaatan Tumbuhan dalam Pengobatan Tradisional Masyarakat Suku Dayak Kanayatn di Desa Ambawang Kecamatan Kubu Kabupaten Kubu Raya [Utilization of Plants in Traditional Medicine of Dayak Kanayatn Tribe Community in Ambawang Village, Kubu District, Kubu Raya Regency]. Jurnal Protobiont, 4(3), 49-59.

Farhatul, B.W. (2013). Potensi Tumbuhan Obat di Area Kampus II UIN Alauddin Samata Gowa [The Potential of Medicinal Plants in the Campus II Area of UIN Alauddin Samata Gowa]. Teknosains: Media Informasi Sains dan Teknologi, 7(1), 111-119. Makasar: UIN Press.

Febrianti, Y., and Krisnawati, Y. (2021). Analisis Pengetahuan Masyarakat Tentang Pemanfaatan Tumbuhan Obat Famili Solanaceae Di Kecamatan Tugumulyo [Analysis of Community Knowledge on the Utilization of Solanaceae Family Medicinal Plants in Tugumulyo District]. Bioma: Jurnal Biologi Makassar, 6(2), 10-22.

Howay, M., Sinaga, N.I., and Kesaulija, E.M. (2003). Utilization of Plants as Traditional Medicines by Maibrat Tribe in Sorong. Beccariana, 5(1): 24-34.

Ismiyanti, N., Diana, A.M., Rizqiyah, S., and Syafiullah, A.M. (2021). Studi Etnofarmasi Tumbuhan Obat Di Desa Pecoro Kecamatan Rambipuji Kabupaten Jember [Ethnopharmaceutical Study of Medicinal Plants in Pecoro Village, Rambipuji District, Jember Regency]. VEKTOR: Jurnal Pendidikan IPA, 2(2), 106-116.

Lesmana, H., Alfianur, A., Utami, P.A., Retnowati, Y., and Darni, D. (2018). Pengobatan tradisional pada masyarakat Tidung kota Tarakan: study kualitatif kearifan lokal bidang Kesehatan [Traditional medicine in the Tidung community of Tarakan city: a qualitative study of local wisdom in the field of Health]. Medisains, 16(1), 31-41.

Linda, R., and Lovadi, I. (2013). Etnobotani Tumbuhan Obat oleh Suku Dayak Iban Desa Tanjung Sari Kecamatan Ketungau Tengah Kabupaten Sintang [Ethnobotany of Medicinal Plants by Iban Dayak Tribe, Tanjung Sari Village, Central Kemite District, Sintang Regency]. Jurnal Protobiont, 2(3).

Marwati, M., and Amidi, A. (2019). Pengaruh budaya, persepsi, dan kepercayaan terhadap keputusan pembelian obat herbal [The influence of culture, perceptions, and beliefs on herbal medicine purchasing decisions]. Jurnal Ilmu Manajemen, 7(2), 168-180.

Meliki, Riza, L., and Irwan, L. (2013). Etnobotani Tumbuhan Obat oleh Suku Dayak Iban Desa Tanjung Sari Kecamatan Ketungau Tengah Kabupaten Sintang [Ethnobotany of Medicinal Plants by Iban Dayak Tribe, Tanjung Sari Village, Central Kemite District, Sintang Regency]. Jurnal Protobiont, 2(3): 129-135.

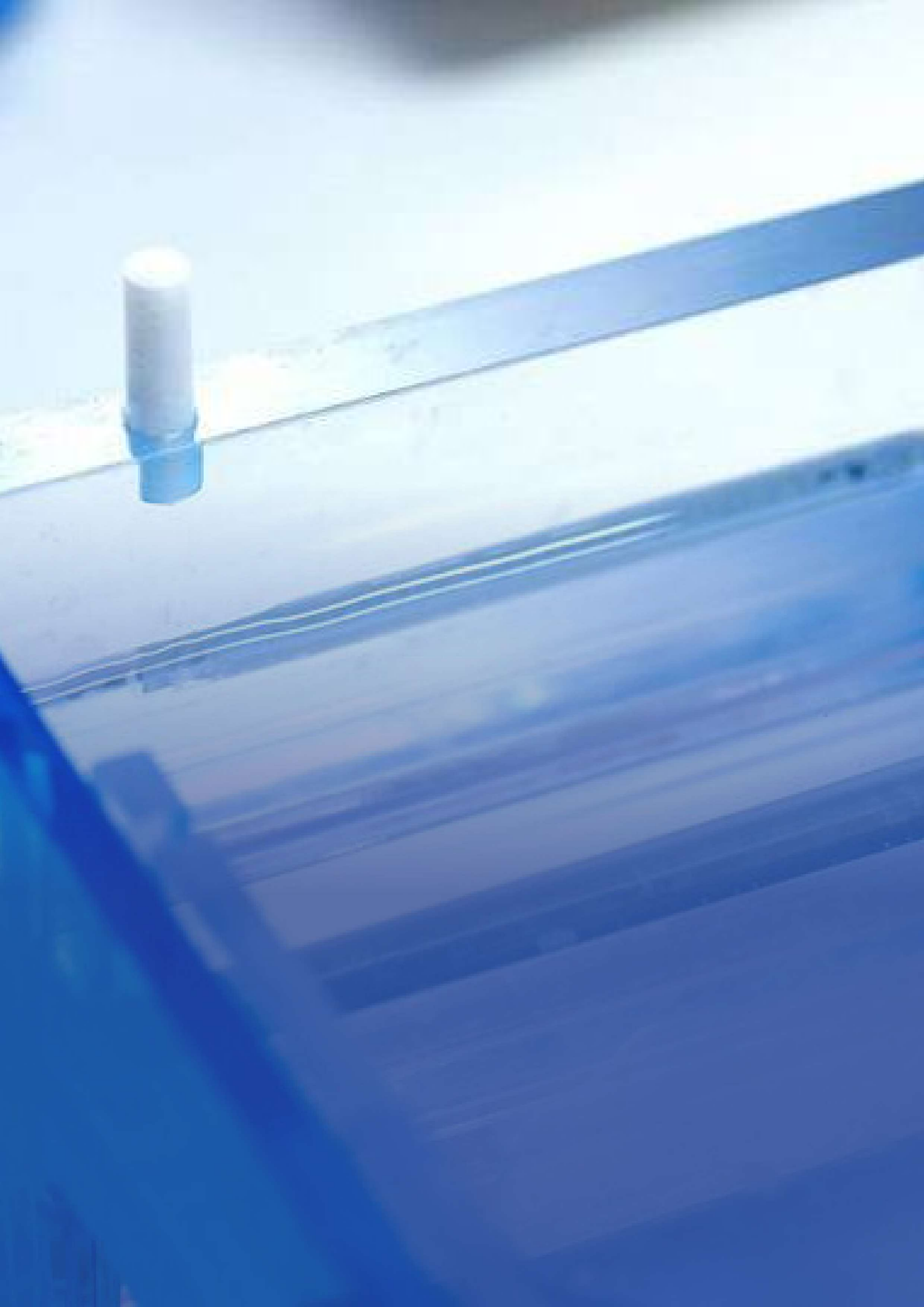
Nulfitriani, Pitopang, R., and Yuniati, E. (2013). Pemanfaatan tumbuhan sebagai obat tradisional pada suku Tolitoli di desa Pinjan Sulawesi Tengah [The use of plants as traditional medicine in the Tolitoli tribe in Pinjan village, Central Sulawesi]. Biocelebes, 7(2), 1-8.

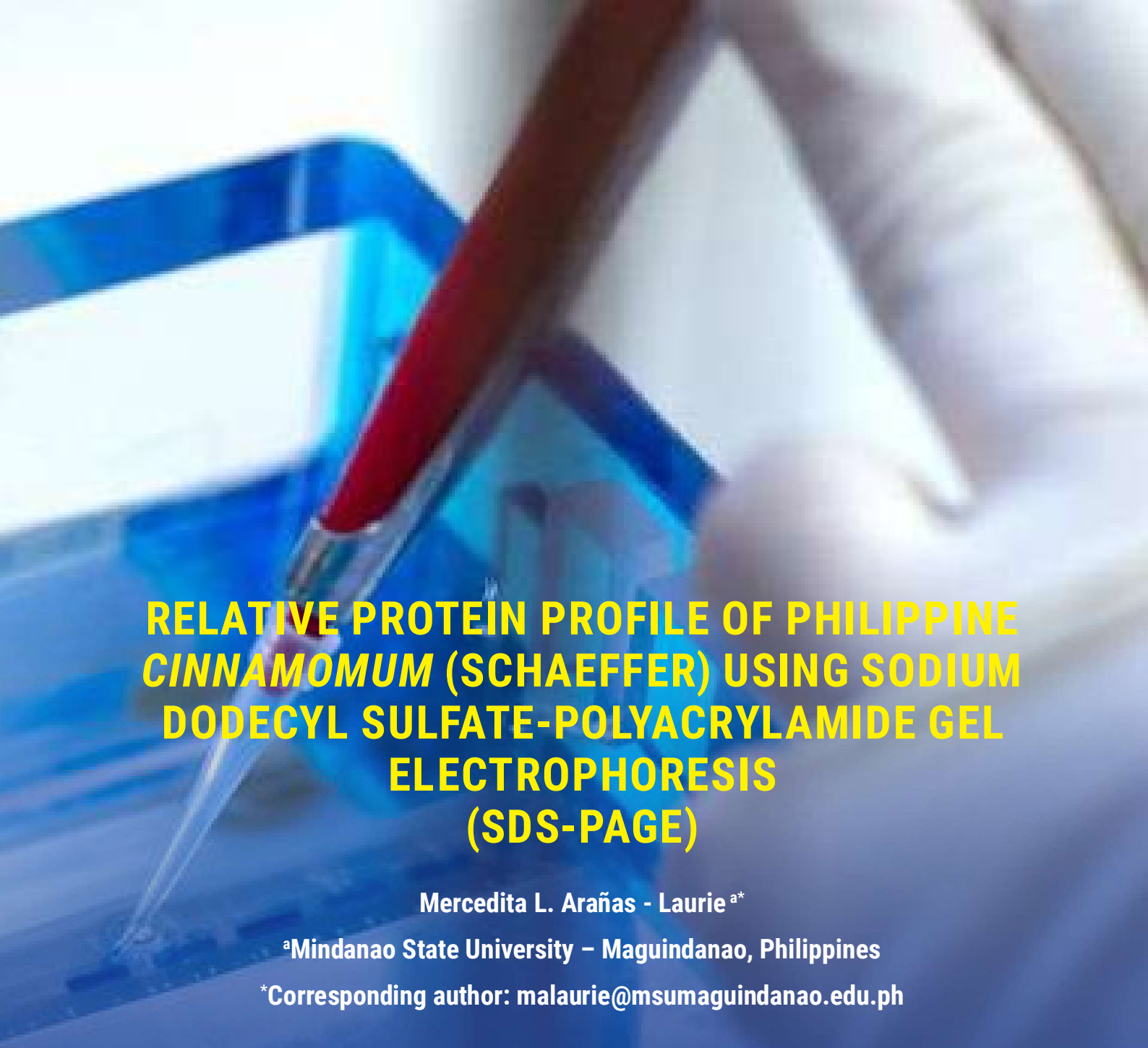
Rusmina, H.Z., Miswan, M., and Ramadhanil, R. (2015). Studi etnobotani tumbuhan obat pada masyarakat Suku Mandar di Desa Sarude Sarjo Kabupaten Mamuju Utara Sulawesi Barat [Ethnobotanical study of medicinal plants in the Mandar Tribe community in Sarude Sarjo Village, North Mamuju Regency, West Sulawesi]. Biocelebes, 9(1): 73-87.

Utomo, D.H. (2017). Etnobotani tumbuhan obat oleh perempuan suku Osing di Kecamatan Glagah Kabupaten Banyuwangi [Ethnobotany of medicinal plants by Osing tribal women in Glagah District, Banyuwangi Regency]. (Doctoral dissertation, Universitas Islam Negeri Maulana Malik Ibrahim).

Wulandara, F.D, Rafdinal, and Linda, R. (2018). Etnobotani Tumbuhan Obat Suku Melayu Desa Durian Sebatang Kecamatan Seponti Kabupaten Kayong Utara [Ethnobotany of Malay Medicinal Plants Durian Sebatang Village, Seponti District, North Kayong Regency]. Jurnal Protobiont, 7(3): 36-46.

Yaqin, A.A. (2020). Etnobotani Tumbuhan Obat oleh Masyarakat Kawasan Taman Nasional Baluran Kabupaten Situbondo [Ethnobotany of Medicinal Plants by the People of Baluran National Park Area, Situbondo Regency]. (Doctoral dissertation, Universitas Islam Negeri Maulana Malik Ibrahim).





RELATIVE PROTEIN PROFILE OF PHILIPPINE *CINNAMOMUM* (SCHAEFFER) USING SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

Mercedita L. Arañas - Laurie [a*]

[a]Mindanao State University – Maguindanao, Philippines

[*]Corresponding author: malaurie@msumaguindanao.edu.ph

ABSTRACT

Philippine Cinnamomum are trees with aromatic leaves and bark. It has several economic and medicinal uses, but concerns about protein profile are limited. Thus, this study was conducted to determine the relative protein component of the species by Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis (SDS-PAGE). A total of 27 Cinnamomum species were run in Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis (SDS-Page) with LSB (Laemli Sample Buffer) and Tris HCl (1:1) as sample buffer. Determining protein profiles is a tool for the systematic classification of the species. Results showed that Philippine Cinnamomum leaf samples have the same component proteins. The SDS-PAGE provided a similar protein banding pattern, although the same bands' intensity varies. Pronounced protein bands were observed corresponding to 50 to 75 kDa and 25 kDa, with additional minor bands indicating a higher protein content. The results in SDS-PAGE showed that the component proteins of Cinnamomum leaves were comparable regardless of geographical source and different stages of growth of the samples. However, the concentration of some component proteins may vary, as indicated by the difference in intensity of protein bands. Sodium dodecyl sulphate Polyacrylamide gel electrophoresis (SDS-PAGE) is a tool in the biochemical method of determining the relative protein profile of Cinnamomum species. It can be used to classify plants up to the species level. Based on the study results, Philippine Cinnamomum has a higher protein content.

Keywords: Philippine Cinnamomum Schaeffer, Relative Protein Profile, SDS-PAGE.

INTRODUCTION

Cinnamomum is a genus of evergreen aromatic trees and shrubs belonging to the laurel family, Lauraceae. The species of Cinnamomum have aromatic oils in their leaves and bark. The genus contains over 300 species distributed in tropical and subtropical regions of North America, Central America, South America, Asia, Oceania, and Australasia. The genus includes many economically important trees (Ravindran et al., 2003).

Cinnamomum has several uses, like spices, flavorings, preservatives, perfume, and soap; some are for liniment and insecticides. Cinnamomum is mostly a folkloric medicinal plant that can cure or relieve several sicknesses and illnesses. It also has anti-fungal (Liu et al., 2001), antibacterial (Kumar et al., 2009), antiviral, and antiseptic properties (Mustapha et al., 2011) and reduces the growth of leukemia and lymphoma cancer cells, colon cancer, and melanoma (Wondrak et al., 2010).

Due to heavy exploitation and deforestation, Cinnamomum plants have been depleted, and some species are placed in the threatened category. Only a few studies have been conducted on protein analysis of Cinnamomum in the Philippines. Relative Protein Profile on Cinnamomum was done through Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for protein analysis to solve these gaps. The results of these investigations can serve as a database for the Relative Protein Profile and be additional data for the correct identification of Philippine Cinnamomum.

The study aims to determine the relative protein profile of Cinnamomum species collected in different mountains in the Philippines. It would provide additional information on the relative protein profile of the Philippine Cinnamomum. Furthermore, the results would be helpful as a basis for policy formulation by the Protected Area Management Board (PAMB) of DENR and the Local Government Unit (LGU) for protecting and conserving the remaining species of Cinnamomum in the Philippines.

METHODOLOGY

This research study includes relative components of the leaf protein of Cinnamomum collected in different places and mountains in the Philippines. The areas of collection of Cinnamomum plants were limited to the following locations: 1) Mindanao: Davao del Sur (Mt. Apo); Socsargen (Mt. Matutum), Davao Oriental (Mt. Hamiguitan); Misamis Occidental (Oroquieta); Misamis Oriental (Naawan); Camiguin Island; Bukidnon (Mt. et al.); Lanao del Sur (MSU-Marawi), 2) Visayas: Negros Oriental (Mt. Guitabon, Mt. Mahayag, and Mt. Kang-Ontol, surroundings of Balinsasayao Twin Lake) and Alcoy, Cebu, 3) Luzon: UPLB Campus and Mt. Makiling.

The laboratory activities were conducted at the Natural Science Research Center (NSRC) Laboratory at Central Mindanao University, Musuan Bukidnon.

Materials used are Silica, dried cinnamon leaf samples, SDS-PAGE Chamber and gel caster, Gel Glass plates and cover, Voltage regulator, Vortex, Centrifuge, Pipettes, Tips and tubes, and Steam bath.

Chemicals used are Deionized water, Methanol, Acetic acid analytical grade, Polyacrylamide, TEMED, APS, Tris HCL, BME, Glycerol, Comassie blue, and Polyacrylamide.

SDS – PAGE BUFFERS:

1.5 M Tris, pH 8.8 (For resolving gel) 250 ml: Cc x N x Mc = grams; Tris, 250 x 1.5 x 0.121 = x; X = grams of Tris needed; X = 45.375 g; Dissolve this to 250 ml with deionize

ed water; Measure pH to 8.8.

1.0 M Tris-HCl, pH 6.8 (For stacking gel); 100 ml: CC x N x Mc = grams Tris; 100 x 1.0 x 0.121 g = x; X = grams of

Tris needed; X = 18.1 g; Dissolve this to 100 ml deionized water. Measure pH to 6.8 with HCl CC1 x N1 = CC2 x N2; 100 x 0.1 = CC2 x 0.121; 10/12 = 0.833 ml ; 833 ul of HCl 99.167 ml deionized water + 833 ul HCl.

1.0 M Tris-HCl, pH 6.8 (For stacking gel); 100 ml: CC x N x Mc = grams Tris; 100 x 1.0 x 0.121 g = x; X = grams of Tris needed; X = 18.1 g; Dissolve this to 100 ml deionized water. Measure pH to 6.8 with HCl CC1 x N1 = CC2 x N2; 100 x 0.1 = CC2 x 0.121; 10/12 = 0.833 ml ; 833 ul of HCl 99.167 ml deionized water + 833 ul HCl.

EXTRACTION BUFFER:

(1:1 LSB with BME and Tris HCl); 7.6 ml (2X Laemli Extraction buffer) + 400 ul BME + 8 ml 50 mM Tris HCl; 2X Laemli Extraction buffer/2 X Sample buffer: (125 mM Tris pH 6.8 + 10% Glycerol + 0.05 % BPB + 0.4 M ME + 2% SDS).

RESOLVING GEL

(12% Polyacrylamide); 30(X) = 12 x 10; X = 4 ml polyacrylamide; 8 ml – good for 2 gels; 2 ml Deionized water; 2.5 ml (1.5 M Tris HCl pH 8.8); 3.3 ml Polyacrylamide (30 %); 100 ul = 0.1 ml 10 % SDS; 4 ul TEMED; (VORTEX – after 20 minutes add); 0.1 ml 10 % APS; (VORTEX and LOAD 3.5 ml per glass plates in a caster); Add 300 ul methanol on top for smooth and even surface; Wait 30 min - 1 hr. for the gel to hardened; Rinse resolving gel with water before adding the stacking gel.

STACKING GEL 4 ml:

2.7 ml Deionized water; 0.5 ml – 1.0 M Tris HCl pH 6.8; 0.67 ml acrylamide solution (Polyacrylamide 30 %); 4 ul TEMED; (Vortex); 40 ul APS (10 %) : add this upon loading; LOAD on top of resolving gel carefully. Install comb, cover with parafilm to avoid dehydration.

0.5 L Staining solution: 200 ml Methanol; 250 ml Deionized water; 50 ml Glacial acetic acid;0.75 g Comassie blue; Mix for at least 4 hrs.; Filter; Store in the dark (cover with aluminum foil 0.5 L Destaining solution: 200 ml Methanol; 250 ml Deionized water; 50 ml Glacial acetic acid.

Flow of Activities:

Field collection, initial identification; preparation of samples/ silica dried; preparation of materials and chemical reagents; laboratory activities; mix reagents for resolving gel, load in glass plates set in gel caster mix reagents for stacking gel, load on top of resolving gel; set glass plates with gel on sds-page chamber; grinding of leaf samples 15-20 mg/tube; add 150 - 200 ul of extraction buffer; vortex; rest for 30 min; waterbath samples in 95Co for two min.; centrifuge at 5 min, 13000 rpm set glass plates with gel on sds-page chamber; Load 15 ul ladder; 15 ul supernatant on wells; RUN in SDS-PAGE Chamber with 1x running buffer at 120 V for 45 min. Remove gel in glass plates carefully, stain, and distain till bands appear clearly, take a picture & laminate the gel. Analyze and interpret the results.

RESULTS AND DISCUSSION

Leaf samples of Cinnamomum from different areas and mountains in the Philippines were collected. A total of 55 samples corresponding to 27 species of collected Cinnamomum were analyzed by Sodium Dodecyl Sulphate – Poly Acrylamide Gel Electrophoresis (SDS-PAGE) with LSB (Laemli Sample Buffer) and Tris HCl (1:1) as sample buffer. Determining protein profile is a tool for the systematic classification of the species.

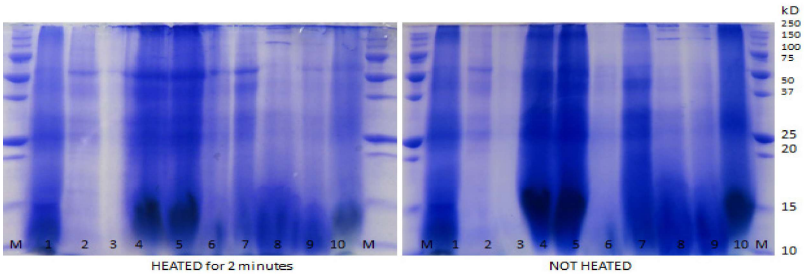


Figure 1. SDS-PAGE (12%) of Cinnamomum cebuense leaf samples at different stages of growth. (M-Molecular weight marker (broad); Lane 1-5 mature age, Lane 6-10 juvenile) Comparisons between heated and non-heated samples.

Figure 1 shows that heated samples at boiling temperature for 2 minutes have more distinct and clear bands of each species' protein component than nonheated ones. It showed that heating gave more resolved protein bands. Heating more likely separates the volatile essential oil in Cinnamomum from its component proteins. Heated treatment exhibited a clear and pronounced band, which was carried throughout the study

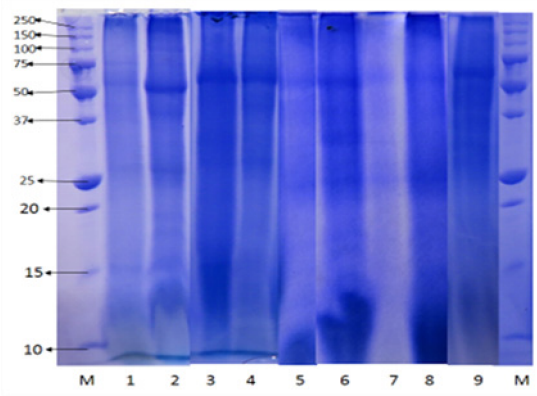


Figure 2. SDS-PAGE (12%) *Cinnamomum* leaf samples from different geographic source. M - Molecular weight marker (broad); 1. *C. sp*.5; 2. *C. mercadoi* (Mis. Occ.); 3. *C. mindanaense* (Bukidnon); 4. *C. oblongum* (Mt. Hamiguitan); 5. *C. loheri*; 6. *C. rupestre*; 7. *C. grifithii*; 8. *C. mercadoi* (Mt. Hamiguitan); 9. *C. sandkuhlii*

Figure 2 shows that the component proteins of all the samples of Cinnamomum are comparable, with distinct bands between 37 kDa and 75 kDa, with minor bands at 12 kDa to 25 kDa. The component proteins are similar, except that some bands are more intense than others.

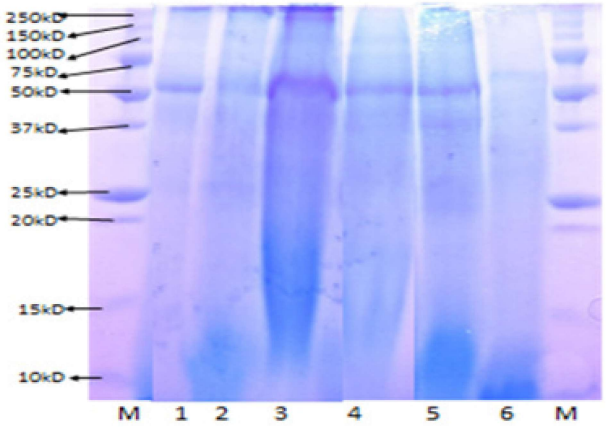


Figure 3. SDS-PAGE (12%) of *Cinnamomum* leaf samples from different geographic source. M - Molecular weight marker (broad); 1. *C. iners* (Bukidnon); 2. *C. mindanaense* (Lanao Sur); 3. *C. burmanni* (S. Cotabato); 4. *C. camphora* (Bukidnon); 5. *C. oblongum* (Bukidnon); 6. *C. sancti-caroli* (Mt. Kiamo, Bukidnon).

Figure 3 shows that the component proteins of all the samples of Cinnamomum are comparable with distinct bands between 50 kDa and 75 kDa, with minor bands at 25 kDa to 37 kDa and 100 kDa to 250 kDa. The component proteins are similar, except that some bands are more intense than others.

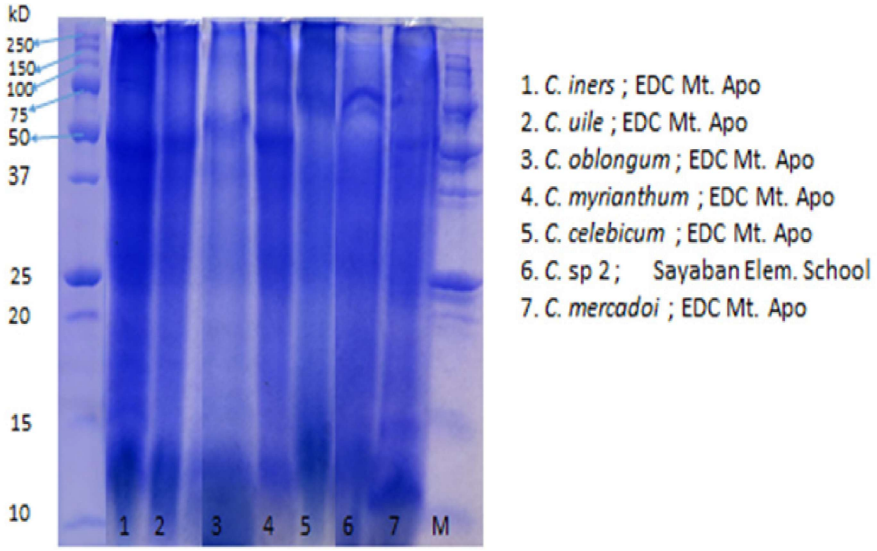


Figure 4. SDS-PAGE (12%) of *Cinnamomum* leaf samples from Mt. Apo. M - molecular weight marker (broad), 1. *C. iners;* 2. *C. utile;* 3. *C. oblongum;* 4. *C. myrianthum;* 5. *C. celebicum; 6. C.* sp.2*;* 7. *C. mercadoi.*

Figure 4 shows that distinct bands of all samples collected at Mt. Apo are at 25 kDa, 50 kDa, and 75 kDa. Minor bands are at 12kDa to 15 kDa and 100kDa to 250 kDa. The component proteins are almost similar, except that some bands are more intense than others.

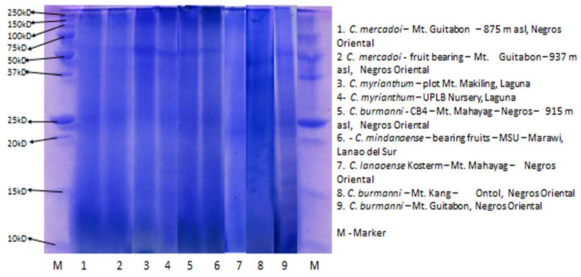


Figure 3. Analysis of Total protein content on SDS-PAGE (12%) showing the variable protein levels of Cinnamomum leaf samples.

Figure 5. SDS-PAGE (12%) of *Cinnamomum* leaf samples from different geographical sources. (M - molecular weight marker (broad), 1. *C.* sp.4 (875 m asl Negros Oriental); 2. *C.* sp.4 (937 m asl Negros Oriental); 3. *C.* sp.7*;* 4. *C.* sp.6*;* 5. *C. ebaloi* (915 m asl Mt. Guitabon Negros); 6. *C. mindanense* (Lanao del Sur); 7. *C. ebaloi* (Mt. Mahayag, Negros); 8. *C. ebaloi* (Mt. Kang-ontol, Negros); *9. C. ebaloi* (Mt. Guitabon, Negros).

Figure 5 shows that the component proteins of all the samples of Cinnamomum are comparable, with distinct bands between 25 kDa and 50 kDa, with minor bands at 12 kDa to 20 kDa and 150 kDa to 250 kDa.

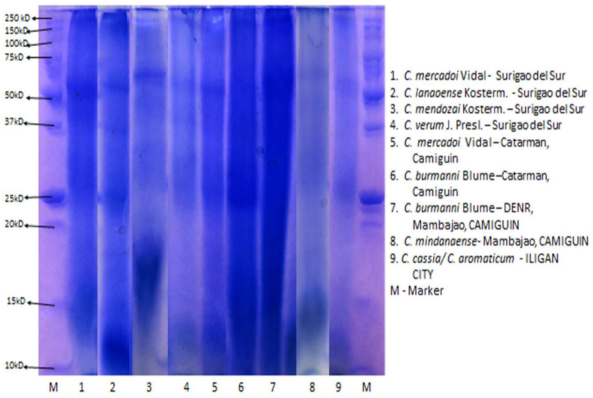


Figure 6**.** SDS-PAGE (12%) of *Cinnamomum* leaf samples from different geographical sources. M - Molecular weight marker (broad); 1. *C. mercadoi* (Surigao); 2. *C. slanaoense;* 3. *C. mendozai;* 4. *C. verum;* 5. *C.* sp.3*; 6. C. burmanni* Catarman Camiguin; 7. *C. burmanni* (Mambajao Camiguin); 8. *C. mindanaense* (Camiguin); 9. *C. cassia*.

Figure 6 shows that the component proteins of all the samples of Cinnamomum are comparable, with distinct bands between 25 kDa and 75 kDa, with minor bands at 12 kDa to 15 kDa and 150 kDa to 250 kDa.

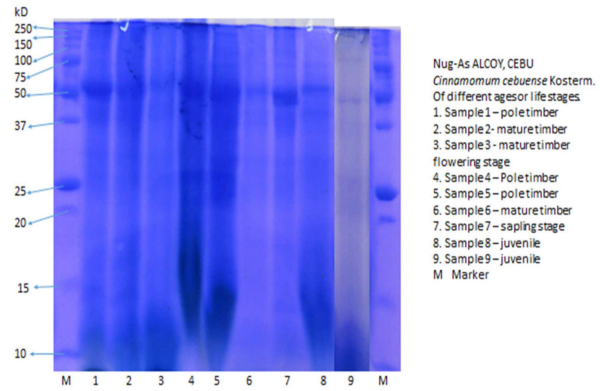


Figure 7**.** SDS-PAGE (12%) of *Cinnamomum cebuense* leaf samples at different stages of growth. (M - molecular weight marker (broad), Lane 1-5 are matured samples, Lane 6-9 are juvenile samples)

Figure 7 shows that the component proteins of all the samples of Cinnamomum are comparable with distinct bands at 37 kDa to 50 kDa, with minor bands at 25 kDa to 37 kDa and 150 kDa to 250 kDa. The component proteins are the same, except that some bands are more intense than others. The results in SDS-PAGE show that the component proteins of Cinnamomum leaves are comparable regardless of geographical source and different stages of growth of the samples. However, the concentration of some component proteins may vary, as indicated by the difference in intensity of protein bands.

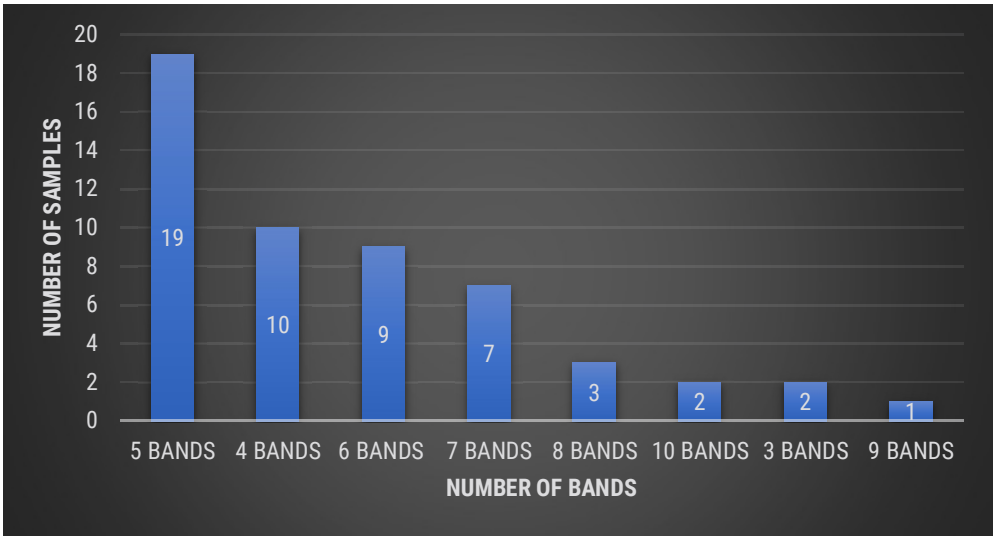


Figure 8. The number of protein bands intensity

Figure 8 shows that 5 bands has the highest number of samples (19), followed by 4 bands with 10 samples, 6 bands with 9 samples, 7 bands with 7 samples, 8 bands with 3 samples, 10 and 3 bands with 2 samples each, and 9 bands has only 1 sample.

Discussion

Sodium dodecyl sulphate polyacrylamide gel electrophores (SDS-PAGE) are the most widely used technique to separate proteins from complicated samples of the mixture. It plays a key role in molecular biology and a wide range of subfields of biological research. In an electric field, when proteins are negatively charged with SDS, proteins migrate toward the negative anode. In SDS-PAGE, the detergent SDS and a heating step determine that the electrophoretic mobility of a single kind of protein is only affected by its molecular weight in the porous acrylamide gel (www.assay-protocol.com).

Determining relative component proteins is a tool for systematically classifying the species. Pragati et al. (2013) also investigated the phylogenetic relationship of nine Ipomoea species, seed proteins were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was used by Kamel and Loufy (2001) in the taxonomy of the Lauraceae. SDS-PAGE of seed protein criteria in the Lauraceae was confirmed.

This study took several times to extract protein due to gelatinous substances from the leaves, and some heating time was optimized. The best result was from partially dried leaf samples and a steam bath of the extracted samples at 95Co. If the bands are distinct, the protein quantity of 50kD has 750 ng, 20kD, and 100 kD has 150 ng each. These values should be used only to determine a rough approximation of the amount of a protein of interest. Almost all species of Cinnamomum have bands of 20 kD, 100 kD, and 50 kD, which means that Cinnamomum in the Philippines has high protein content.

CONCLUSIONS

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a biochemical tool for determining the relative protein profile of Cinnamomum species. All species of Cinnamomum have similar protein content but differ only in intensity. Based on the result of the study, Philippine Cinnamomum has a higher protein content.

ACKNOWLEDGMENT

Heartfelt thanks and gratitude to Dr. Annabelle P. Villalobos of Johnsons and Johnsons Company USA, Dr. Victor B. Amoroso Director of Center of Biodiversity Research and Extension in Mindanao (CEBREM), Central Mindanao University (CMU), Musuan Bukidnon, Dr. Fulgent P. Coritico, Dr. Lowell G. Aribal, Ms. Angie Rose Villafranca Registered Chemist and Mr. Rainer Mendez Registered Chemist.

REFERENCES

Kumar , A.B.S., K. Lakshman, S.M. Tripathi, K.N. Jayaveera, V.B. Swamy, K. Saleemila and R.R. Nandeesh. (2009). Free Radical Scavenging and Antibacterial Activities of Amry-card Powder (Ayurvedic Formulation). The Internet Journal of Alternative Medicine. 17(1): 1-8.

Liu, C.H., A.K. Mishra, B. HE, and R.X. Tan. (2001). Composition and antifungal activity of essential oils from Artemisia princeps and Cinnamomum camphora. International Pest Control. 43(2): 72-74.

Mustaffa F., Indurkar, J., Ismail S., Shah, M. and Mansor SM. (2011). An antimicrobial compound isolated from Cinnamomum iners leaves with activity against methicillin-resistant Staphylococcus aureus. Molecules. 16(4):3037-47.

Pragati, V.G. P. and Sreenath, K.P. (2013). Phylogenetic Relationship of Some Ipomoea Seed Proteins by SDS-PAGE. International Research Journal of Biological Sciences, India. ISSN 2278-3202. Vol.2(10), 64-67.

Ravindran, P. N., K. N. Babu; M. Shylaja (2003). Cinnamon and Cassia: The genus Cinnamomum. CRC Press. p. 59. ISBN 978-0-415-31755-9.

Wondrak, G., Thomas, N.F., Villeneuve, S.D., Lamore, A.S., Bause, T., Jiang, D.D. and Zhang, D. (2010). The Cinnamon-Derived Dietary Factor Cinnamic Aldehyde Activates the Nrf2-Dependent Antioxidant Response in Human Epithelial Colon Cells. NCBI. 15(5):3338-3355