Gas Chromatography-Mass Spectrometry (GC-MS) Analysis for the Identification of Antifungal and Antioxidant in Tobacco Waste Extracts

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Abstract. Tobacco waste (*Nicotiana tabacum* L.) is the final product produced by the tobacco industry. Tobacco waste contains the same potential bioactive compounds as tobacco leaves. Tobacco waste can be processed into environmentally friendly biopesticides because it contains bioactive compounds with antifungal and antioxidant activities. This research can help farmers in agricultural engineering by utilizing agricultural products that produce waste. Research was conducted to identify bioactive compounds present in tobacco waste extract using *Gas Chromatography-Mass Spectrometry* (GC-MS) method. The method used was the extraction of tobacco waste with ethanol solvent followed by a maceration process by *Ultrasonic Assisted Extraction* (UAE) to produce optimal bioactive compounds. The UAE method with a temperature of 60ºC and time of 30 minutes can produce higher bioactive compounds. GC-MS identification showed a total of 40 bioactive compounds, with the highest component being *3-(1-methyl-2-pyrrolidinyl)pyridine* at 86.07% area percentage. This compound is called nicotine with the main alkaloid content present in tobacco waste extract. This compound has toxic ingredients that inhibit and control pests on plants. Tobacco waste extract contains antifungal with a value of 85.79% and antioxidant activity (IC_{50}) shows a value of 14.30 ppm.

Keywords: Antifungal, Antioxidant, GCMS, Tobacco Waste

1. Introduction

Tobacco is a leading commodity that plays an important role in Indonesia in the agricultural, economic, and social sectors. Tobacco produces waste consisting of parts that are not used in the tobacco industry and tobacco that is not qualified [1]. According to the Ministry of Agriculture of the Republic of Indonesia in 2020, the land area of tobacco plants reached a total of 204,961 ha, with a range of tobacco plant population per hectare of land of 22,000 trees and a weight of about 0.5 kg. In total, tobacco tree waste reached 2,302,268.36 metric tons [2]. The potential for solid waste from tobacco processing is 14–20% of the raw material for cigarette making in the form of tobacco handle residue, tobacco grinding and sieving waste, and clove handle residue [3]. Waste generated by cigarette companies is reported to be 15 trucks per day or 15 tons per month [4].

Tobacco waste can be converted into high-value products. The waste is processed into compost, biopesticides, bio-oil, bio-char, bio-gas, and biodiesel [5]. Tobacco waste from the cigarette industry contains nicotine active compounds as much as 2%, which has the potential to be a biopesticide in inhibiting plant-destroying insects [6]. Tobacco waste containing nicotine functions as an insecticide and fungicide because it acts as a

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acts as a contact poison to control several types of leaf-damaging caterpillars, soft-bodied sucking insects, and fungi [7].

Tobacco waste can be processed into biopesticides that can act as contact antifungals for controlling caterpillars or pests in plants. One of the pests in cayenne pepper is the fungus *Colletotrichum gloeosporioides*. This disease is a member of the *colletotrichum* genus and can cause damage and crop losses of up to 100% [8]. Fungal spores can be spread by wind, splashing rainwater, and attached to suitable hosts that can develop quickly. Wet plant surface conditions affect the germination of fungal spores, the infection process, and the growth of pathogens on host plants [9]. Anthracnose disease is characterized by the presence of blackish-brown spots on the surface of the fruit. At the center of the spot is a collection of black dots consisting of a group of setae and conidia of the fungus [10].

Tobacco waste is processed into biopesticides using maceration extraction and *Ultrasonic Assisted Extraction* (UAE). UAE is an efficient technique that promotes high extraction yields but sometimes low selectivity. The use of ultrasonic methods is the same as that of microwaves, which pass through media by creating compression and expansion. Ultrasound produces a phenomenon called cavitation. Cavitation is the main phenomenon shaping the technique, where ultrasonic waves form bubbles in the solvent and consequently cause the rupture of the dissolving matrix of molecules in the extraction solution [11]. Time and temperature efficiency in the UAE are used to optimize the bioactive compounds present in plant extracts [12], [13]. Maceration and UAE extraction are needed in processing tobacco waste to maximize the content of bioactive compounds that inhibit fungal growth and have antioxidant content that functions as a biopesticide.

2. Research Methodology

2.1. Research Time and Place

The research was conducted from July to September 2023 at the Industrial Design and By-product Control Laboratory, Bioindustry Laboratory, Food Chemistry and Biochemistry Laboratory of Agricultural Products, Faculty of Agricultural Technology and Integrated Research and Testing Laboratory (LPPT), Gadjah Mada University, Yogyakarta, Indonesia.

2.2. Research Materials and Tools

The materials used were tobacco waste (Jember), *Colletotrichum gloeosporioides* fungus (Yogyakarta), ethanol, methanol, acetone, 10% DMSO, Potato Dextrose Agar, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The tools used were IKA rotary evaporator RV 10 digital V, IKA membrane vacuum pump MVP 10 basic, IKA control temperature RC 2 L, ultrasonic processor UP200St,and column GC-MS HP-5MS UI.

2.3. Sample Preparation

Tobacco waste was obtained from unused and unqualified tobacco leaves. Tobacco waste was sorted and dried in an oven at 50 °C. The material was blended and sieved to a 40-mesh size so that the particle surface was the same. Tobacco waste powder was dissolved in 96% ethanol solvent in a ratio of 1:10. The solution was stirred using an ultrasonic processor to break down the bioactive ingredients for 30 minutes at 60

°C [14]. Then the solution was macerated for 24 hours. The result of maceration was filtered, and the supernatant was re-macerated for 24 hours. The filtrate was evaporated using a rotary vacuum evaporator for 50 minutes. The concentrated extract was then dried with a cabinet dryer at 50 °C. Then the extract was tested for GC-MS, antifungal, and antioxidant properties [15].

2.4. Analysis of Extracts

2.4.1. GC-MS analysis

The paste sample was dissolved in ethanol solvent and homogenized by centrifugation at 9500 rpm for 3 minutes. The sample was loaded into the HP-5MS UI column. The GC was operated at 60 °C for 2 minutes, then raised to 280 °C in 100 °C increments. The total gas flow rate used was 50 ml per minute with a split ratio of 1:50, an injector temperature of 230 °C, and an amount of sample injected into the injector of 0.1 µl [16], [17].

2.4.2. Antifungal Activity

Prepare Potato Dextrose Agar (PDA) media on a petri dish by adding tobacco waste extract that has been dissolved in 10% DMSO. Wait until the media solidifies, then add the inoculum of the *Colletotrichum gloeosporioides* fungus. The media that has been inoculated is incubated at 25 °C for 7 days [18], [19]. Then the diameter of the fungal colonies that grow on the medium is calculated.

$$D = \frac{DK-DP}{DK} x\ 100\% \quad (1)$$

D, antifungal activity (%). DK, diameter of the mushroom colony growing in the negative control (cm). DP, diameter of the mushroom colony that grew in the treatment (cm)

2.4.3. Antioxidant Activity

Prepare 5 mg of a tobacco waste extract sample dissolved with methanol p.a. 100 ml into a sample concentration of 500 ppm (mother solution). From the parent solution, dilutions were made with concentrations of 50 ppm, 75 ppm, 100 ppm, and 125 ppm. Then each concentration was taken into 2 ml and added to 2 mL of DPPH (0.1 mM). The mixture was homogenized with a vortex and incubated for 30 minutes in a dark room, then tested for absorbance value using a UV-Vis spectrophotometer at a wavelength of 517 nm [20], [21].

Antioxidant activity was interpreted by the DPPH method with an efficient concentration (EC_{50}) value commonly called IC_{50}. The IC_{50} value was calculated from the inhibition of 50%y = ax+b with the following inhibition formula:

$$\%\ inhibition = \frac{absorbance\ of\ the\ blank - sample\ absorbance}{blank\ absorbance} x\ 100\% \quad (.2)$$

3. Result and Discussion

3.1. GC-MS Analysis

Gas Chromatography-Mass Spectrometry is a combined separation technique to identify chemical compounds in samples [22]. Results separation by gas chromatography results in a chromatogram, while the results of mass spectrometry examination of each compound is called a spectrum [17].

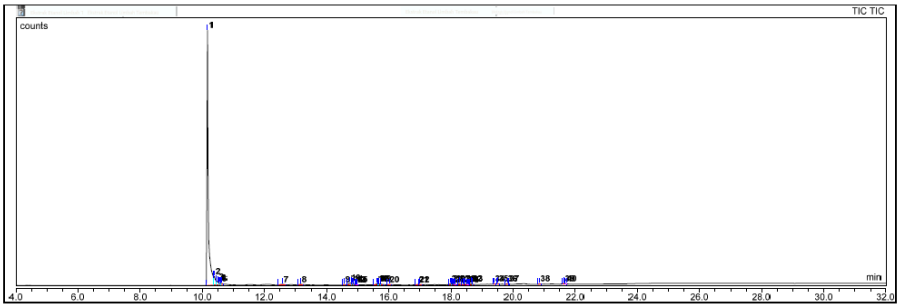


Figure 1. Kromatogram GC-MS Tobacco Waste Extract

The identification results show that there are 40 peaks and 40 possible compound components contained in tobacco waste extract with ethanol solvent samples. Peak 1 at a retention time of 10.17 minutes, with the highest resistance area value at 86.07%. Peak 1 spectrum is shown in Figure 2, which shows the highest compound content of tobacco ethanol extract. The compounds contained is *Pyridine, 2-(1-methyl-2-pyrrolidinyl); Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S);* and *Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, 1-oxide, (S)*, with the chemical formulas $C_{10}H_{14}N_2$ and $C_{10}H_{14}N_2O$. This compound is an organic compound found in tobacco, namely nicotine. The spectrum also shows the molecular weight of nicotine compounds, which is 162,1 m/z; this is in accordance with the characteristics of nicotine [23].

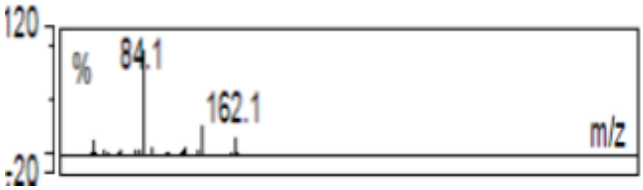


Figure 2. Spectrum Nicotine Compound Tobacco Waste Extract

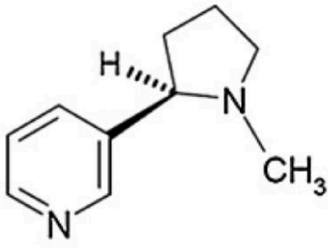


Figure 3. Structure Nicotine Compound [23]

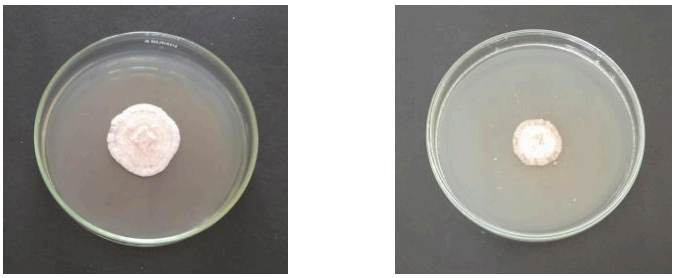
The main form of nicotine in nature is in the S-(-) isomer. (S)-nicotine binds stereoselectively to nicotinic cholinergic receptors (nAChRs) (Benowits, 2009). Nicotine is the main alkaloid content present in tobacco extract, along with pyrrolidine and pyridine rings. Nicotine is an organic compound that generally consists of carbon, hydrogen, nitrogen, and oxygen, with a concentration of about 5% of the weight of tobacco [23].

Tobacco waste extract showed GC-MS results at a retention time of 12.761 minutes containing nicotine with an area of 49.18% [24]. The area of nicotine content is the highest among other components. The GC-MS results of nicotine content in tobacco leaf liquid smoke were located at a retention time of 15.482 minutes and an area of 45.82% [25]. The GC-MS results of nicotine content in cigarette butt waste are located at a retention time of 8.292 minutes and an area of 71.85%, which is the main component in insecticides [26].

Ethanol extracts of tobacco waste carried out have differences with the literature. This is because the nicotine content is influenced by the main base material, namely tobacco leaves or waste. In addition, the type of tobacco can affect the content and nicotine levels. The area produced on nicotine is influenced by temperature on GC-MS, the higher the temperature used, the lower the area produced on nicotine [27].

3.2. Antifungal Activity

Ultrasonic Assisted Extraction (UAE) is an effective technique for the extraction of bioactives from plant substances. The mechanochemical effects of ultrasound can accelerate the extraction process due to damage to the cell wall and increased mass transfer of cell contents without changing the structural and molecular properties of the plant [28]. The time and temperature efficiency in the UAE are used to optimize the bioactive compounds present in plant extracts [12], [13].



(a) (b)

Figure 4. (a) Negative control inhibition, (b) inhibition by tobacco waste extract treatment

The diameter of the control treatment was 4.55 cm, and the diameter of the tobacco waste extract treatment was 0.65 cm. The antifungal activity of the tobacco waste extract was 85.79%. The stirring that occurs in the UAE maceration method increases the extraction of compounds that contain high nicotine. These compounds contain antifungals that can inhibit fungal growth [29]. Nicotine in tobacco plants is a toxic

material that can be used as an insecticide, fungicide, acaricide, or molluscicide that works as a contact poison in the stomach and acts as a fumigant that will evaporate and also penetrate directly into the integument. Nicotine is the main component of nicotinic insecticides and insect repellent mechanisms [30].

Antifungal activity in tobacco waste has very strong inhibitory power [18]. Tobacco ethanol extract in research Duan [14] produced antifungal values of 50–70%. This antifungal functions by inhibiting the growth of the *Colletotrichum gloeosporioides* fungus. Nicotine compounds contained in tobacco waste function as contact poisons for fungi. This is in accordance with pesticides that are usually used to inhibit cayenne pepper fungi of the ditio carbamate or *mankozeb* group, which function as contact poisons [31].

3.3. Antioxidant Activity

Antioxidants are electron donor compounds that can inhibit oxidation reactions by binding free radicals and highly reactive molecules. Antioxidants counteract free radicals in the body so that they can fight oxidative damage and also inhibit the oxidation process of fat or oil so that it has a function as a preservative [32]. Antioxidants are active ingredients found in every plant and can be used as biopesticides [33].

The antioxidant activity of tobacco waste extract was tested using the DPPH method with UV-Vis spectrophotometry at a maximum wavelength of 517 nm. The amount of antioxidant activity is indicated by the IC_{50} value, which is the concentration of sample solution required to inhibit 50% of DPPH free radicals. The IC_{50} value of the extract was determined using the linear regression equation of the curve of the relationship between sample concentration and percent inhibition with the equation Y = ax + b, sample concentration (ppm) as the axis (X), and the percentage inhibition as the (Y) axis [34].

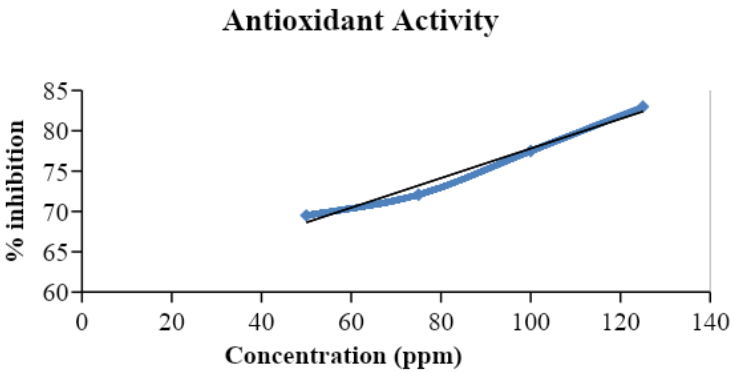


Figure 5. Relationship Curve Tobacco Waste Extract Yield Against Inhibition Percentage

The antioxidant activity produced from tobacco waste extract is 14.30 ppm. This value is the IC_{50} value, where a low IC_{50} value indicates strong antioxidant activity.

Antioxidants produced from tobacco waste extracts have very strong activity because they have an IC_{50} value of less than 50 ppm [35]. Antioxidants in tobacco waste are influenced by the presence of flavonoids and phenol compounds that have superoxide anions that can counteract free radicals [36].

Tobacco waste extract using ethanol solvent has the smallest IC_{50} value because there are bioactive compounds that are nonpolar and semipolar, so polar solvents (ethanol) attract more bioactive components in tobacco waste [34]. The ethanol extract of tobacco waste has 20% inhibition with an IC_{50} value of 66–230 ppm [21]. This shows that there is a difference in the IC_{50} value, which is influenced by the extraction process. Prommaban [21] research, using maceration extraction for 48 hours. While in this study, using UAE extraction and maceration for 24 hours. So that it can produce a smaller IC_{50} value with higher antioxidant activity.

4. Conclusion

Tobacco waste can be processed into biopesticides by maceration extraction and *Ultrasound Assisted Extraction* (UAE). This biopesticide contains nicotine, which functions as an antifungal with 85.79% inhibition. In addition, it has a very strong antioxidant content with an IC_{50} value of 14.30 ppm.

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References

[1] A. Wardhono, J. A. Arifandi, and Y. Indrawati, "Standar dan Mutu Tembakau Besuki Na-Oogst," in *CV. Pustaka Abadi*, Jember: CV. Pustaka Abadi, 2019, p. 101.

[2] R. R. Amin, R. R. Sova, D. I. Laily, and D. K. Maharani, "Artikel Review Studi Potensi Limbah Tembakau Menjadi Bio-Oil Menggunakan Metode Fast-Pyrolysis Sebagai Energi Terbarukan," *J. Kim. Ris.*, **vol. 5, no. 2**, pp. 151–165, 2020.

[3] Y. S. Ningsih, "Uji Efektivitas Ekstrak Limbah Tembakau Sebagai Pestisida Nabati Terhadap Ulat Helicoverpa Armigera (Lepidoptera: Noctuidae) Pada Jagung," *Univ. Brawijaya*, vol. Skripsi, 2019.

[4] DPR RI, "Pengelolaan Limbah di Perusahaan Rokok PT . HM Sampoerna," *Kunjungan Spesifik Komisi VII DPR RI*, vol. Sekretariat, 2018.

[5] Kementerian Kesehatan, "Bunga Rampai : Fakta Tembakau dan Permasalahannya di Indonesia," in *Tobacco Control Support Center*, 2014, p. 190.

[6] A. L. Kusumawardhani, Basir, Subandriyo, and Nilawati, "The Use Solid Waste Of Cigarette Industry For Bio-Pesticide Up-Flow Anaerobic Sludge Blanket (Uasb) Process For Better Performance And Cost Efficiency Of Waste Water Treatment Of Paper Industry," *J. Ris. Teknol. Pencegah. Pencemaran Ind.*, **vol. 2, no. 1**, pp. 21–28, 2012.

[7] R. Khalalia, "Uji Daya Bunuh Granul Ekstrak Limbah Tembakau (Nicotianae

Tabacum L) Terhadap Larva Aedes Aegypti," *Unnes J. Public Heal.*, **vol. 5, no. 4**, p. 366, 2016, doi: 10.15294/ujph.v5i4.11844.

[8] W. Anggraeni and E. R. P. Wardoyo, "Isolasi dan Identifikasi Jamur Pada Buah Cabai Rawit (Capsicum frutescens L .) Yang Bergejala Antraknosa Dari Lahan Pertanian Di Dusun Jeruk," *Protobiont,* **vol. 8, no. 2**, pp. 94–100, 2019.

[9] P. P. Than, R. Jeewon, K. D. Hyde, O. Mongkolporn, and P. Taylor, "Characterization and pathogenicity of Colletotrichum species associated with anthracnose on chilli (Capsicum spp .) in Thailand Characterization and pathogenicity of Colletotrichum species associated with anthracnose on chilli (Capsicum spp .) in Thaila," *Plant Pathol. J.*, **vol. 57**, pp. 567–572, 2008, doi: 10.1111/j.1365-3059.2007.01782.x.

[10] B. S. Weir, P. R. Johnston, and U. Damm, "Studies in Mycology : The Colletotrichum gloeosporioides species complex," *Stud. Mycol.*, **vol. 73**, pp. 115–180, 2012, doi: 10.3114/sim0011.

[11] L. S. Contieri *et al.*, "Recent Progress On The Recovery Of Bioactive Compounds Obtained From Propolis As A Natural Resource: Processes, And Applications," *Sep. Purif. Technol.*, **vol. 298,** no. July, 2022, doi: 10.1016/j.seppur.2022.121640.

[12] M. Oroian, F. Dranca, and F. Ursachi, "Comparative Evaluation Of Maceration, Microwave And Ultrasonic-Assisted Extraction Of Phenolic Compounds From Propolis," *J. Food Sci. Technol.*, **vol. 57, no. 1**, pp. 70–78, 2020, doi: 10.1007/s13197-019-04031-x.

[13] T. Sirichan, I. Kijpatanasilp, N. Asadatorn, and K. Assatarakul, "Optimization Of Ultrasound Extraction Of Functional Compound From Makiang Seed By Response Surface Methodology And Antimicrobial Activity Of Optimized Extract With Its Application In Orange Juice," *Ultrason. Sonochem.*, **vol. 83**, p. 105916, 2022, doi: 10.1016/j.ultsonch.2022.105916.

[14] S. Duan *et al.*, "Chemical Basis Of The Fungicidal Activity Of Tobacco Extracts Against Valsa Mali," *Molecules*, **vol. 21, no. 12**, 2016, doi: 10.3390/molecules21121743.

[15] S. Al-Lahham *et al.*, "Antioxidant, Antimicrobial And Cytotoxic Properties Of Four Different Extracts Derived From The Roots of Nicotiana tabacum L.," *Eur. J. Integr. Med.*, **vol. 33,** no. December 2019, p. 101039, 2020, doi: 10.1016/j.eujim.2019.101039.

[16] W. Y. Teoh *et al.*, "LC-MS / MS and GC-MS Analysis for the Identification of Bioactive Metabolites Responsible for the Antioxidant and Antibacterial Activities of Lygodium microphyllum," *Separations*, **vol. 10**, pp. 1–12, 2023.

[17] N. Nurhaen, D. Winarsii, and A. Ridhay, "Isolasi dan Identifikasi Komponen Kimia Minyak Atsiri dari Daun, Batang dan Bunga Tumbuhan Salembangu (Melissa sp.)," *Nat. Sci. J. Sci. Technol.*, **vol. 5, no. 2**, pp. 149–157, 2016, doi: 10.22487/25411969.2016.v5.i2.6702.

[18] N. Diana, S. Khotimah, and Mukarlina, "Penghambatan Pertumbuhan Jamur Fusarium oxysporum Schlecht Pada Batang Padi (Oryza sativa L.) Menggunakan Ekstrak Metanol Umbi Bawang Mekah (Eleutherine palmifolia Merr.)," *Protobiont*, vol. 3, no. 2, pp. 225–231, 2014.

[19] J. B. Sinclair and O. D. Dhingra, *Basic Plant Pathology Methods*, vol. 2nd

Editio, no. https://doi-org.ezproxy.ugm.ac.id/10.1201/9781315138138. 2019.

[20] D. Tristantini, A. Ismawati, B. T. Pradana, and J. Gabriel, "Pengujian Aktivitas Antioksidan Menggunakan Metode DPPH pada Daun Tanjung (Mimusops elengi L)," *Pros. Semin. Nas. Tek. Kim. "Kejuangan,"* p. 2, 2016.

[21] A. Prommaban, K. Kheawfu, C. Chittasupho, S. Sirilun, K. Hemsuwimon, and W. Chaiyana, "Phytochemical, Antioxidant, Antihyaluronidase, Antityrosinase, and Antimicrobial Properties of Nicotiana tabacum L. Leaf Extracts," *Evidence-based Complement. Altern. Med.*, vol. 2022, 2022, doi: 10.1155/2022/5761764.

[22] I. G. Gandjar and A. Rohman, "Kimia Farmasi Analisis," in *Pustaka Belajar*, 2009. Yogyakarta.

[23] S. Chulurks, "Preservation of Nicotine and Solanesol From Tobacco by Cyclodextrins Nanoencapsulation," *Thesis*. Sirindhorn, 2020.

[24] K. Yati, "Skrining Fitokimia Ekstrak Daun Tembakau (Nicotiana Tabaccum L .) Dan Aktivitasnya Terhadap Candida albicans," *Univ. MUHAMMADIYAH PROF. DR. HAMKA*, pp. 1–38, 2019.

[25] Kuntjahjawati and P. Darmaji, "Identifikasi Komponen Volatil Asap Cair Daun Tembakau (Nicotina tabacum L.) Rajangan," *Agritech*, vol. 24, no. 1. pp. 17–22, 2004.

[26] F. O. Erian, A. Muarif, N. ZA, Z. Ginting, and Zulnazri, "Pemanfaatan Ekstrak Nikotin Dari Limbah Puntung Rokok Menjadi Insektisida," *J. Teknolo*, **vol. 11, no. 2**, pp. 258–266, 2022.

[27] R. Muzyka, M. Chrubasik, M. Dudziak, M. Ouadi, and M. Sajdak, "Pyrolysis Of Tobacco Waste: A Comparative Study Between Py-GC/MS And Fixed-Bed Reactors," *J. Anal. Appl. Pyrolysis*, **vol. 167**, no. September, p. 105702, 2022, doi: 10.1016/j.jaap.2022.105702.

[28] X. shi Yang *et al.*, "Antioxidant Activities Of Polysaccharide Fractions Isolated From Burley Tobacco Flowers," *Process Biochem.*, **vol. 5, no. 12**, pp. 217–224, 2013, [Online]. Available: http://dx.doi.org/10.1016/j.carbpol.2010.07.045%5Cnhttp://dx.doi.org/10.1016/j.procbio.2012.02.004.

[29] M. Banožić, J. Babić, and S. Jokić, "Recent Advances In Extraction Of Bioactive Compounds From Tobacco Industrial Waste-A Review," *Ind. Crops Prod.*, **vol. 144**, no. December 2019, 2020, doi: 10.1016/j.indcrop.2019.112009.

[30] F. Nouri, S. R. Nourollahi-Fard, H. R. Foroodi, and H. Sharifi, "In Vitro Anthelmintic Effect Of Tobacco (Nicotiana tabacum) Extract On Parasitic Nematode, Marshallagia Marshalli," *J. Parasit. Dis.*, vol. 40, no. 3, pp. 643–647, 2016, doi: 10.1007/s12639-014-0550-3.

[31] T. K. Meokasan, L. Prabaningrum, and W. Adiyoga, "Cara Kerja dan Daftar Pestisida serta Strategi Pergilirannya pada Budidaya Tanaman Sayuran dan Palawija," *vegIMPACT*, no. December, pp. 6–24, 2014.

[32] A. L. Ningsi, "Aktivitas Antioksidan Dari Ekstrak Daun Tembakau (Nicotiana tabacum L.) Yang Berasal Dari Desa Cabbenge Kabupaten Soppeng," *Skripsi*, Makassar, Universitas Islam Negeri Alauddin, 2018.

[33] N. Hartini, S. Richana, B. Triwibowo, N. Qudus, and R. Dewi, "Sintesis Nanoenkapsulasi Ekstrak Kulit Durian dengan Metode Spray Drying dan

Aplikasinya sebagai Biopestisida : Review," *J. Tek. Kim. dan Lingkung.*, **vol. 2, no. 2**, pp. 89–95, 2018.

[34] D. Purwanto, S. Bahri, and A. Ridhay, "Uji Aktivitas Antioksidan Ekstrak Buah Purnajiwa (Kopsia arborea blume.) Dengan Berbagai Pelarut," *Kovalen*, **vol. 3, no. 1**, p. 24, 2017, doi: 10.22487/j24775398.2017.v3.i1.8230.

[35] P. Molyneux, "The Use Of The Stable Free Radical Diphenylpicryl-Hydrazyl (DPPH) For Estimating Antioxidant Activity," *Songklanakarin J. Sci. Technol.*, **vol. 50**, no. June 2003, pp. 211–219, 2004.

[36] S. Ben Nasr, S. Aazza, W. Mnif, and M. Miguel, "Phenol Content And Antioxidant Activity Of Different Young And Adult Plant Parts Of Tobacco From Tunisia , Dried At 40 And 70 ^{O}c," *J. Appl. Pharm. Sci.*, **vol. 4, no. 08**, pp. 23–31, 2014, doi: 10.7324/JAPS.2014.40805.