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The Volatile Compound Profiles of Fire-Cured and Fermented Na-Oogst Tobacco Leaves (*Nicotiana tabacum* L.) and Its In-Silico Study

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Abstract: Na-Oogst tobacco is one of the local commodities of Jember, which has been widely used as a raw material for making cigarettes. Product diversification in processing tobacco leaves is very necessary to reduce the danger caused by cigarette consumption. It is also an effort to empower and save the economy of tobacco farmers. Tobacco leaf' essential oil could be an alternative in maintaining tobacco production. The purpose of this study is to determine the profile (yield and chemical compounds) of Na-Oogst tobacco leaf' essential oil content in pre- and post-fermentation. Extraction of essential oils is carried out by two methods, i.e., steam distillation and maceration. The results showed that the extraction from the maceration method produced higher yields than from steam distillation, while the extract from tobacco leaves before fermentation was lower than that after fermentation. The major identified compounds found in tobacco leaves from GC-MS analysis are neophytadiene, nicotine, ledol, phytol, and solanone. Neophytadiene is suggested as a marker compound in tobacco leaf since it is present in all extracts and essential oils in significant amounts, about 23.72-67.37% of the total identified compounds. Further molecular docking of neophytadiene against the D2 dopamine receptor is also investigated in this study.

Keywords: extraction, essential oil, fermentation, tobacco.

INTRODUCTION

Na-Oogst type of tobacco, also known as Besuki Na-Oogst, is one of the local commodities in the Jember area that has been widely used for certain needs. Most of the people of Jember use this type of tobacco as a raw material in the manufacture of cigarettes and cigars, and have not diversified. Cigarettes generally contain dangerous components, such as nicotine, nitrosamine, and benzopyrene. Therefore, it increases public awareness of health issues and raises some government policies in reducing cigarette production, banning smoking in public places, and promoting an incessant anti-smoking campaign. Meanwhile, tobacco farmers still need to plant and distribute tobacco crops to fulfil their daily needs.

Extraction of essential oils is an alternative effort in maintaining a sustainable tobacco production. Tobacco leaves' essential oil, containing aromatic components, has several benefits, including as an active ingredient for antiseptic, anti-inflammatory, analgesic, antioxidant, and antimicrobial [1]. Essential oils also show high economic opportunities to be marketed, both domestically and abroad. The export value of Indonesian essential oils increased significantly from 2015 to 2016. According to Indonesian Ministry of Trade, total exports of essential oil in 2015 reached US$ 637.4 million and increased to US$ 694.7 million in 2016.

The volatile compounds in the tobacco leaf's essential oil may be influenced by the environment and its development stage. It was assumed that essential oils are produced more in plants with sufficient sunlight. In addition, the environmental factors such as air temperature, humidity, water content, and mineral composition also affect the volatile oil content in a plant. Another important factor affecting the chemical content of essential oils in plant leaves is the post-harvesting process, such as drying and fermentation. The kind of drying process for tobacco leaves consists of air-curing, sun-curing, flue-curing, fire-curing, and oven-drying [2]-[4]. Each method gives different characteristics to the aroma and flavour of tobacco. Fermentation for tobacco leaves occurs in both natural and artificial types. Natural fermentation involves stacking leaves in a pile, generating natural heat and humidity, while artificial fermentation controls the temperature and humidity by placing the leaves in barrels or kilns [5]. At this stage, some additives can be added to the process to enhance the quality of tobacco leaves, such as citrus flavonoids [6].

The process of extracting essential oils in plants can be done by several extraction methods, such as soxhlet extraction, percolation, maceration, reflux, and distillation. The distillation method can separate the essential oil content in tobacco leaves by 0.02-0.063% of dry weight and identify them further by gas chromatography (GC) [7]. The main content of essential oils in tobacco leaves consists of solanone (29.5%) and neophytadiene (23.0%) [1]. Another method that can be used for extracting tobacco leaves' essential oil is solvent extraction through the maceration process.

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Tobacco leaves have been successfully extracted by the maceration method using ethanol, resulting 19.55% to 31.35% yield of extract [8], [9].

This research has a main aim of identifying the influence of tobacco leaves' fermentation on the chemical content of their essential oils. The comparison of chemical profiles between tobacco leaf' essential oils and extracts is also analysed in this paper. This research has a long-term purpose to maintain the existence of the tobacco plantation by providing a potential alternative application in producing essential oil and extract for medical needs, perfume, and cosmetic industries.

METHODS

Materials

Na-Oogst tobacco leaves were obtained from PT. Mangli Djaya Raya, Jember. The tobacco leaves consisted of the original post-harvested leaves or cured-tobacco leaves (labelled as: pre-fermentation) and those after fermentation for 4 months (labelled as: fermentation). These leaves were checked for their moisture content based on AOAC 1995 and then crushed into powder form before being used for extraction.

Steam Distillation

The powder of Na Oogst tobacco leaves, about 200 g, was put in a 1 L three-neck round-bottom flask, and water was added until three-quarters of the flask volume. The steam was produced from another flask containing 4 L of water acting as a boiler, which is connected to the sample flask with a glass pipe. The samples were distilled for 9 hours, with three repetitions. The distillation results (essential oils) were collected and then weighed to calculate the yield. Whenever the oil still contained water, anhydrous $MgSO_4$ was added to absorb the water. The dried essential oils were kept in a cool place before further analysis.

Maceration

The tobacco leaves powder was extracted with non-polar solvent, i.e, n-hexane. About 150 g of tobacco leaves powder was soaked in 600 mL of n-hexane for 24 hours. The filtrate was separated from the residue using a Buchner filtration funnel. The filtrate was collected in an erlenmeyer, while the residue was repeatedly immersed in n-hexane for the next 24 hours. These treatments were repeated three times, and the filtrate from each extraction was collected together in an erlenmeyer flask. The combined filtrate was evaporated further using a rotary evaporator and then weighed to obtain the yield value. The extract was kept in a cool place before use.

Analysis of the Extracts and Essential Oils

The extracts and essential oils were determined for the yield value based on the amount of the product obtained from the dry weight of the samples. Further detailed analysis was the identification of chemical components of Na-Oogst tobacco leaves extracts and essential oils using *Gas Chromatography-Mass Spectroscopy* (GC-MS). The sample was injected into the GC-MS to mix with the mobile phase (carrier gas) of Helium. The injection temperature used was 300°C and the column temperature was 70°C. The separated components from the GC column were then sent to the detector, Mass Spectrometer (MS). The components were bombarded with electrons 70 eV until further fragmentation occurred and recorded based on their m/z value. The chromatogram and mass spectrum were combined to perform a valuable analysis containing retention times, percentage area, and chemical compound names.

Statistical Analysis

The GC-MS data of chemical components from the essential oils and extracts were analysed statistically using Principal Component Analysis (PCA) and a Venn diagram.

Molecular Docking

The molecular docking study was carried out using AutoDock Tools software to generate grids, calculate binding affinity and RMSD (Root Mean Square Deviation). The centre grid box was determined first from interaction of the protein target and native ligand (Risperidone), with the centre on the ligand set up to produce RMSD less than 2 A°. The dimension of the grid (x x y x z) was in 40 x 40 x 40 with a specific position on 9.177 x 5.47 x -8.665, respectively. The conversion of each format file, such as Mol2, pdb, and pdbqt formats, was facilitated using OpenBabel. The genetic algorithm parameters were run for 50 iterations, population size as much to 300 solutions (chromosomes), and the number of evaluations was in a medium-scale specific operation, 2.500.000 times. The visualisation of 2D and 3D interactions between ligands and protein receptors was facilitated by Biovia Discovery Studio.

RESULT AND DISCUSSION

The post-harvested tobacco leaves are commonly dried before being stored or subjected to the fermentation process. The one presented in PT. Mangli Djaya Raya is post-harvested tobacco leaves that have undergone a further drying process through the fire-cured procedure. Fire-cured is a drying method of tobacco leaves with a low fire from firewood on a closed drying warehouse floor. Smoke from combustion can give a specific aroma and taste to tobacco leaves. This fumigation process aims to reduce the water content in tobacco leaves. The drying process of tobacco leaves is an attempt to reduce the water content to reach the equilibrium moisture content, so it is safer to store. The smoked Na-Oogst tobacco leaves are prepared by piercing its leaves' bone parts with a skewer model and then placing them on top of the tobacco smoke house (Figure 1). The length of time needed for the fumigating process of Na-Oogst tobacco leaves is about a full day and night, with a source of heat coming from burning wood or charcoal. This process causes a change in the chlorophyll of tobacco leaves so that the original colour of the leaves turns yellowish [2]. In the drying process, there is also a conversion of starch into sugar and the removal of leaf moisture. This process can give rise to certain aromas and flavours in every variation of drying method on tobacco leaves. Further discussion will label this kind of tobacco leaves as fire-cured tobacco leaves or tobacco leaves before fermentation.

Another post-harvest treatment of Na-Oogst tobacco leaves is the one with the fermentation method (Figure 1). The intended fermentation is an advanced process of fumigating tobacco leaves, which has the purpose of increasing the maturity of the leaves [10]. Some processes that occur during fermentation are the condensation of polysaccharides and amino acids through the Maillard reaction, the oxidation of polyphenols to quinone, and the transformation of leaf plastid pigments such as chlorophyll into carotenoids [2]. Those reactions lead to the browning of tobacco leaves and also affect their quality and appearance. The

fermentation process can be regulated by controlling humidity and temperature. Fermentation of Na Oogst tobacco leaves can be done from one to four months with temperatures of approximately 50°C. Fermented tobacco leaves are stacked at a predetermined time and temperature, and then undergo further oper-staple processing. Oper staple is a removal of the pile position of tobacco leaves from the top to the bottom, or vice versa, which further determines the maturity of tobacco leaves. Further discussion in this paper will label this kind of tobacco leaves as fermented tobacco leaves or tobacco leaves after fermentation.

Figure 1. Fermentation of Na-Oogst tobacco leaves at PT. Mangli Djaya Raya, Jember.

Extraction of Chemical Components in Tobacco Leaves

Chemical compounds in both kinds of tobacco leaves (before and after fermentation or pre- and post-fermentation) are extracted with two methods, steam distillation and maceration. Steam distillation is carried out by flowing hot steam from the boiler flask into the plant sample chamber in order to encourage the chemical components inside the sample to be extracted more easily. Volatile components of the samples (in the form of gas molecules) go up to the condenser together with the hot steam water to change their form into liquid. The liquid then runs to a separating funnel and forms two phases, consisting of essential oil in the upper phase and water in the lower phase. The essential oils produced from the steam distillation method have a thick texture and deep brown colour.

Maceration is carried out by soaking dried Na-Oogst tobacco leaves powder in n-hexane for 24 hours at room temperature to break down the cell walls and membranes to create different pressures between inside and outside the cells. Non-polar solvent will dissolve non-polar components in the sample based on the like dissolves like mechanism. Further filtration separates the filtrate and residue of the sample from and solvent. The evaporated filtrate results in a blackish brown concentrated and slightly solidified tobacco leaves extract, which is mostly composed of terpenoids. Tobacco leaf extract is also known as tobacco resinoid, which mostly contains aliphatic hydrocarbons, oxygenated aliphatics, and diterpenes [11], [12].

Table 1. The Yield of Tobacco Leaf Extract and Essential Oil Pre- and Post-Fermentation

Tobacco Leaves Extract	Yield (%)
Maceration	
Before fermentation	3,6991
After fermentation	3,8697
Steam Distillation	
Before fermentation	0,1138
After fermentation	0,2994

There is a significant difference in the yield of extracted tobacco leaves obtained by maceration and steam distillation methods. The yield of the extract from the maceration method is greater than that one from the steam distillation method. It is because the maceration method uses non-polar solvents, n-hexane, which has a polarity index of 0, so that it is able to extract many non-polar compounds present on tobacco leaves. All non-polar compounds, including essential oils are will be well recovered in this solvent, resulting in a high per cent yield of extract. This data is in accordance with other research showing that the solvent extraction of basil leaves using n-hexane obtained a greater yield in terms of equilibrium and kinetics compared to the use of a polar solvent, ethanol. Moreover, this research also proves that the essential oil from the extraction process produces 4 g of oil, while steam distillation produces 1.9 g of oil [13].

However, the yield of tobacco leaves' essential oils and extracts from both samples, pre- and post-fermentation, has quite significant differences (Table 1). The yield of both extracts from tobacco leaves post-fermentation is greater than that pre-fermentation. This fact is influenced by the post-harvest treatment, i.e., fermentation, of the tobacco leaves. Tobacco leaves experiencing the fermentation process show a higher level of maturity, causing an increase in their secondary metabolite contents. This result is in accordance with previous research on patchouli oil extraction, showing that the yield of essential oils from the samples pre- and post-fermentation (14 days) process has a significant difference, 6 mL/kg and 14 mL/kg, respectively [14]. The leaves from the pre-fermentation method undergo a curing process, which does not maximise the maturity level of the leaves and results in less content of their chemical components. In addition, higher water content in the leaves will prevent the release of chemical components during extraction processes. Therefore, the yield of extracts from tobacco leaves before fermentation is smaller than those that undergo the fermentation process.

Chemical Composition of the Extracts and Essential Oils from Tobacco Leaves

The tobacco leaf extracts and essential oils show a high diversity of chemical constituents after being identified using GC-MS analysis (Table 2). Total compounds present in tobacco leaves before and after fermentation obtained using the steam distillation and maceration are as many as 71 compounds.

Table 2. Volatile Components of Na-Oogst Tobacco Leaves Essential Oils and Extracts – Before and After Fermentation

No	Compounds	Relative Percentages (%)			
		SD Before	Maceration Before	SD After	Maceration After
1	Solanone	2.08	-	5.46	-
2	Trans-Caryophyllene	0.96	0.21	2.25	0.78
3	Geranylacetate	1.35	-	-	-
4	Farnesol	1.58	-	4.92	-
5	Norsolanadione	0.79	-	-	0.4
6	n-pentadecane	0.81	-	-	-
7	Beta-bisabolene	1.01	-	-	-
8	1-decene	0.95	-	2.57	-
9	Neophytadiene	67.37	30.2	23.92	34.63
10	1-octadecyne	0.94	-	-	-
11	1-tetracosanol	1.11	-	-	-
12	(E)-9-methyl-2-Undecene	1.08	-	-	-
13	Cembrene	1.54	-	-	-
14	1-Hexacosene	1.1	-	-	-
15	Dipentene diepoxide	1.05	-	-	-
16	Diallyl acetal palmitaldehyde	2.05	-	-	-
17	Phytol	3.22	8.65	-	-
18	2-heptadecyl-thiophene	4.95	-	-	-
19	Camphogen	-	0.54	-	-
20	Durene	-	0.38	-	-
21	Nikotin	-	20.53	-	27.94
22	1-tetradecene	-	0.75	1.1	-
23	Undecanal	-	0.31	-	-
24	S-(Z)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol	-	0.32	-	-
25	Metil dekanoat	-	0.22	-	-
26	1R-(1R-,2R-,5S-,6E-10R-)-8-Methylene-5-(1-methylethyl)-spiro-11-oxabicyclo 8.1.0 undec-6-ene-2,2'-oxiran-3-one	-	0.46	-	-
27	1-Heptadecene	-	0.67	-	-
28	17-Acetoxy-19-Kauranal	-	1.69	-	1.3
29	Isopulegol asetat	-	5.83	-	4.83
30	9-Isopropylenylbicyclo(6.1.01-8)nonane	-	0.82	-	0.53
31	Patchulane	-	1.6	1.9	1.04
32	1-Octadecene	-	0.45	-	-
33	Nerolidol-epoxyacetate	-	0.94	-	1.69
34	Nerolidol	-	1.2	-	-
35	Ledol	-	13.7	-	5.17
36	Caryophyllene oxide	-	0.25	0.41	-
37	Lignocerol	-	0.25	-	-
38	Farnesyl acetone	-	0.25	-	-
39	Oleol	-	0.5	-	-
40	Eicosane	-	1.01	-	0.33
41	Dotriacontane	-	0.65	-	1.33
42	Hexatriacontane	-	1.15	-	0.64
43	1,2-Dimethylbenzene	-	-	0.52	-
44	1,3,5,7-Cyclooctatetraene	-	-	0.83	-
45	1,2,3 trimethylbenzene	-	-	1.69	-
46	2,3,6-Trimethyl-1,5-heptadiene	-	-	2.98	-
47	trans-3-Undecene	-	-	1.04	-
48	4-isopropyl-1-methyl-Cyclohexene	-	-	2.51	-
49	Limonene	-	-	9.62	-
50	Trans-Ocimene	-	-	0.61	-
51	n-Undecane	-	-	1.37	-
52	3-Methyl-3-phenylcyclopropene	-	-	0.76	-
53	1-Dodecene	-	-	2.81	-
54	2,6-Dimethylundecane	-	-	0.91	-
55	2,3,7-trimethyl-Octane	-	-	0.81	-
56	n-Dodecane	-	-	5.1	-
57	Solanone	-	-	5.46	-

No	Compounds	Relative Percentages (%)			
		SD Before	Maceration Before	SD After	Maceration After
58	5-propyl-Nonane	-	-	1.33	-
59	1-Dodecene	-	-	1.87	-
60	Hexadecyl chloride	-	-	1.14	-
61	2-ethenyl-2,5-dimethyl-4-Hexen-1-ol	-	-	5.27	-
62	Ledane	-	-	1.28	-
63	Hexadecane	-	-	1.56	-
65	Durene	-	-	-	0.51
66	Methyl 14-methyl-pentadecanoate	-	-	-	0.31
67	Methyl 11-octadecenoate	-	-	-	1.14
68	2,6-dimethyl-1,7-octadiene-3-ol	-	-	-	7.43
69	Di-n-octyl phthalate	-	-	-	4.42
70	Octacosane	-	-	-	0.97
71	3-Methyldodecane	-	-	-	0.55

SD Before = steam distillation - before fermentation
Mace Before = maceration - before fermentation
SD After = steam distillation - after fermentation
Mace After = maceration - after fermentation.

Table 3. Major Components in Tobacco Leaves Essential Oils and Extracts Before and After Fermentation

Compounds	Compound Abundance (%)			
	SD Before	SD After	Maceration Before	Maceration After
Limonene	-	9,62	-	-
Nicotine	-	-	20,53	27,94
Solanone	2,08	5,46	-	-
Farnesol	1,35	3,61	-	-
2-ethenyl-2,5-dimethyl-4-hexen-1-ol	1,58	5,27	-	-
Neophytadiene	67,37	23,92	30,20	34,63
Isopulegol acetate	-	-	5,83	4,83
2,6-dimethyl-1,7-octadiene-3-ol	-	-	-	7,43
Ledol	-	-	13,70	5,17
Phytol	3,22	-	8,65	-
2-heptadecyl-tiophene	4,95	-	-	-

SD = steam distillation

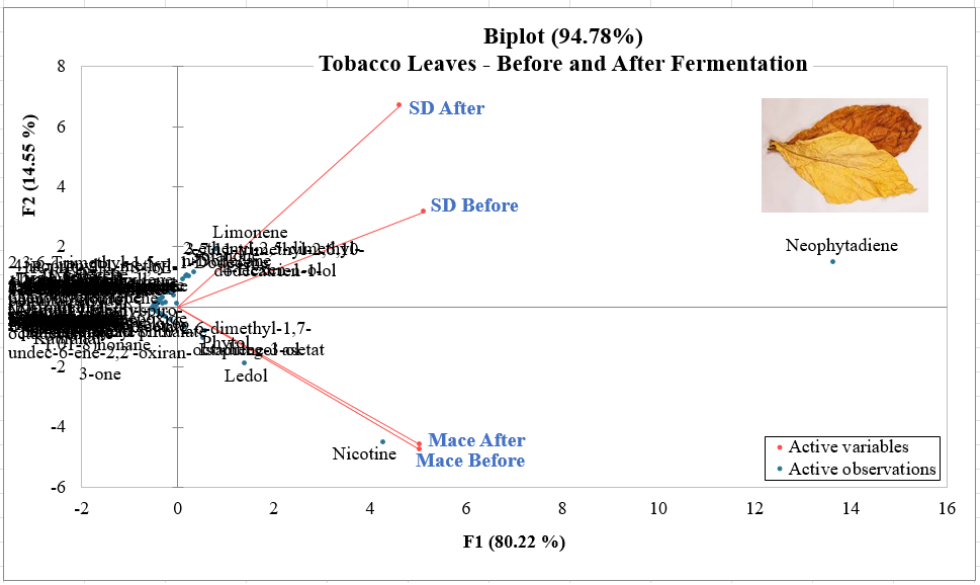

Figure 2. PCA of tobacco leaves chemical compositions identified by GC-MS. SD Before = steam distillation – before fermentation. Mace Before = maceration – before fermentation. SD After = steam distillation – after fermentation. Mace After = maceration - after fermentation.

The composition of major compounds composing essential oils of fire-cured and fermented tobacco leaves obtained from steam distillation and maceration methods is shown in Table 3. Neophytadiene is a typical aroma found in tobacco leaves. This

compound can be noted as a marker compound in tobacco leaves.

The PCA of secondary metabolite content of tobacco leaves extracts and essential oils shows that neophytadiene can be annotated as a marker compound since it is found in significant amounts of tobacco leaves extracts and essential oils (Figure 2). Another component found specifically in the extracts from both tobacco leaf samples is nicotine. This compound does not appear in the essential oil components, showing that nicotine cannot be extracted through distillation since it is not a volatile compound. While limonene and ledol are two chemicals identified significantly present in each extracts and essential oils of the tobacco leaves.

This PCA analysis is supported by the data presented in the Venn diagram (Figure 3). The diagram shows that neophytadiene is one of two components, besides trans-caryophyllene, present in all the extracts and essential oils from tobacco leaves. Nicotine is also one of the common elements found in the extracts of tobacco leaves obtained from the maceration method. Essential oil of steam-distilled tobacco leaves from pre- and post-fermentation has two same major compounds, i.e., solanone and 2-ethenyl-2,5-dimethyl-4-hexen-1-ol. Major compounds that are different between variations, pre- and post-fermentation, are six compounds (Table 3). Limonene, farnesol and tetradecyl oxirane are major compounds that are only found in essential oils of fermented tobacco leaves. While neophytadiene, phytol and 2-heptadesyl-tiofen compounds are only found in fire-cured tobacco leaves' essential oils.

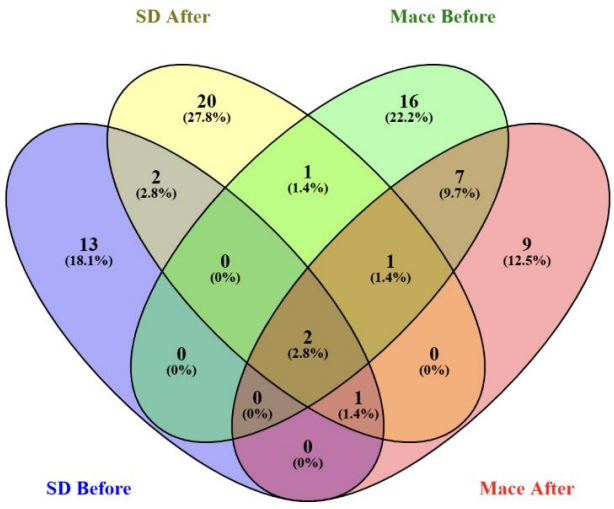

Figure 3. Diagram Venn of the chemical compositions of tobacco leaves essential oils and extracts – before and after fermentation. SD Before = steam distillation – before fermentation; Mace Before = maceration – before fermentation; SD After = steam distillation – after fermentation; Mace After = maceration – after fermentation.

Table 3 provides information that the major essential oils produced by maceration of tobacco leaves after fermentation, with the greatest abundance, are neophytadiene and nicotine. This is in accordance with other research on tobacco leaves maceration using ether, which produced major compounds including neophytadiene, about 0.06-0.1% of dry weight [16], and ultrasound-assisted extraction with hexane produced up to 9.4% neophytadiene and up to 87.5% nicotine [17]. In addition to compounds with greater abundance in tobacco leaves' essential oil after fermentation, there are also compounds with smaller abundance than before fermentation, such as ledol compounds and isopulegol acetate.

Phytol is derived from the hydrolysis of chlorophyll in plants. However, phytol disappeared in both essential oil and extract since it degrades into neophytadiene via dehydration reaction [15]. Rowland has isolated neophytadiene from tobacco leaf and obtained about 0.06-0.1% of dry weight [16]. In this research, neophytadiene is the compound with the greatest abundance in tobacco leaves' essential oil in pre- and post-fermentation, about 67.37% and 41.93%, respectively. This is in accordance with the previous research showed that the largest chemical component in tobacco leaves' essential oil is neophytadiene, with an abundance of up to 74.15%, while solanone abundance is up to 9.55% [18]. Solanone was isolated firstly time from tobacco in 1965 with a yield of 0.0036% [19], [20]. However, this compound can be found up to 7.94% in the essential oil of tobacco from the East Java region [7]. In this research, solanone and its autooxidation product, norsolanadione, are found in the essential oil of tobacco leaves up to 5.46% and 0.79%, respectively. They are normally useful as a tobacco flavorant. This fact is consistent with another research mentioned that solanone is present together with norsoladione in tobacco, with a composition of about 2.68-6.87% and 0.22-0.73%, respectively [21].

Nicotine is known to decrease anxiety and depression, improve cognitive performance, and increase alertness. Nicotine content in flue-cured tobacco was considered to decline compared to fresh-harvested tobacco [2], [22] as the contribution of microorganism activity [23], but this study shows otherwise. Nicotine in tobacco leaf extracts was identified to increase from 20.53% to 27.94% after the fermentation process. Amin et al [22] suggested more about this fact, that the total alkaloid concentration in tobacco leaves after the drying process corresponded to stalk position and harvesting season.

Volatile compounds contribute to the overall aroma profile of tobacco leaves, essential oils and extracts. Nerolidol supports a floral, woody, bark-like scent, and it has been isolated and produced in high yield from agroinfiltrated tobacco [24]. However, in this research, nerolidol and its derivative, nerolidol-epoxyacetate are disappear during fermentation. Other alcoholic compounds are found to be present after fermentation, including 2-ethenyl-2,5-dimethyl-4-hexen-1-ol and 2,6-dimethyl-1,7-octadiene-3-ol. In addition, farnesol rises from 1.58% to 4.92%, while ledol declines significantly from 13.7% to 5.17%, after fermentation. The enhancement of these alcoholic compounds may be a result of the naturally available microorganism activity during tobacco leaf fermentation [25].

Previous research shows that other components, such as lutein, β-carotene, chlorogenic acid, caffequinic acid, rutin, kaempferol glycosides, and polyphenols, are the main compounds responsible for the leaves' colour change during the fermentation stage [26]. Those compounds are non-volatile components that are precisely identified using HPLC or LC-MS. Therefore, the use of another instrument based on liquid chromatography is required instead of GC-MS for a comprehensive analysis of the influence of drying and fermentation methods.

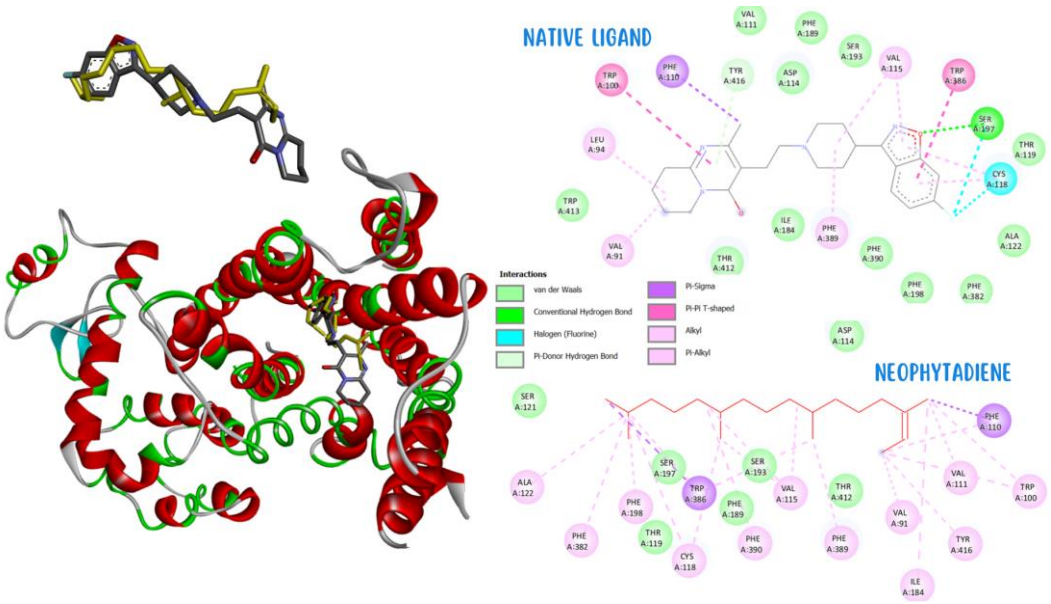

Figure 4. Docking visualization for 3D and 2D interactions between D2 dopamine receptor target (PDB ID: 6CM4) with native ligand (Risperidone) and neophytadiene. Superimposed native ligand and neophytadiene is shown on the top of left corner.

Neophytadiene is a major component present in all kinds of essential oils and extracts from fire-cured and fermented tobacco leaves. This compound gives taste and aroma to tobacco leaves. It also shows anxiolytic-like and anticonvulsant activities that affect the in vivo neuropharmacology of mice [27]. Dopamine receptors have a critical role in the motoric, cognitive, and psicomotoric system controlled in the brain. However, molecular docking simulations of neophytadiene with the D2 dopamine receptor are not extensively documented. Therefore, in this research, neophytadiene is subjected to molecular docking toward the dopamine receptor.

Risperidone is the native ligand that is available as a complex with the dopamine D2 receptor in the PDB website with ID 6CM4 [28]. The binding energy resulted from the interaction between the D2 dopamine receptor target and the native ligand, Risperidone, is -14.53 kcal/mol, while between the protein target and neophytadiene, as the major phytochemical in tobacco leaf, is -8.01 kcal/mol (Figure 4). The binding pocket of neophytadiene in 6CM4 contains of residues Ser197, Trp386, and Phe110 for the interaction of π-σ bonds. It is quite different from the interaction of native ligand with 6CM4, providing hydrogen bonding with residues Ty416 and Ser197, π-σ bonding with Phe110, π-π bonding with Trp100, and halogen interaction with Cys118 and Ser197. The presence of hydrogen bonding in native ligand–protein receptor and halogen interaction results in a higher binding energy value compared to neophytadiene, which has no heteroatoms such as halogen, oxygen and nitrogen. Previously, neophytadiene has also been docked toward other protein targets, such as α-glucosidase (3WY1), α-amylase (1SMD), and sucrose-isomaltase (3LPO) [29]. However, the binding affinity values of those interactions are lower compared to the interaction against the D2 dopamine receptor in this research. Activity of neophytadiene against Human LRH1 LBD (3PLZ) also shows high binding energy about -9.01 kcal/mol [30]. In addition, the binding affinity of neophytadiene against biofilm formation of Staphylococcus aureus (NCBI Acc. No: CPM65595.1) is higher than the standard inhibitor or control drug, Metronidazole [31]. Neophytadiene has been analysed for the ADMET and CYP properties to obey the Lipinski rule of five, has low intestinal absorption, and does not cross the blood-brain barrier [32]. Therefore, application of the essential oil or extract containing neophytadiene should be safe in the perfume or cosmetic industry, with a precaution on its concentration. The summary of this research is simply depicted in Figure 5.

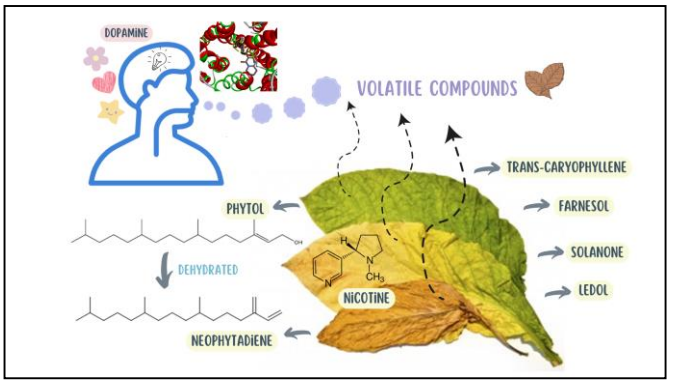

Figure 5. Pictorial representation of the release of volatile components from tobacco leaves, which are then being inhaled by humans to influence dopamine receptor targets within the brain.

CONCLUSION

The research shows that the production of extract and essential oil of fire-cured and fermented tobacco leaves can be an alternative to product diversification other than cigarette production. Fermentation is a way of enhancing the quality of tobacco leaves. The per cent yield of maceration is significantly higher than the essential oil from the distillation method. Fire-cured tobacco leaves produce a lower yield of essential oil than fermented ones. Some major compounds identified in tobacco

leaves are neophytadiene, nicotine, ledol, phytol, solanone and farnesol. Neophytadiene and trans-caryophyllene are two compounds found in all extracts and essential oils of tobacco leaves. According to that fact, neophytadiene can be suggested as a marker compound in tobacco leaves. Furthermore, molecular docking interaction between neophytadiene and D2 dopamine receptor results in binding energy of -8.01 kcal/mol compared to the native ligand, -14.53 kcal/mol. This fact suggests that neophytadiene in tobacco could play an important role as an antidepressant, though its activity is not as much as controlled medicine.

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REFERENCES

[1] S. Alagic, I. Stancic, R. Palic, G. Stojanovic, and M. Nikolic, "Chemical Composition and Antimicrobial Activity of the Essential Oil of the Oriental Tobacco Yaka," *J. Essent. Oil Res.*, vol. 14, pp. 230-232, 2002.

[2] J. Zong *et al.*, "Effect of two drying methods on chemical transformations in flue-cured tobacco," *Drying Technology*, vol. 40, no. 1. pp. 188-196, 2022. doi: 10.1080/07373937.2020.1779287.

[3] V. Radojicic, M. Srbinaska, L. Pejic, and T. Urosevic, "Chemical transformations in flue-cured tobacco depending on the drying method: a review," in *Proceedings of the XV International Scientific Agricultural Symposium Agrosym 2024*, pp. 678-683, 2024.

[4] G. Guo *et al.*, "Characterization of moisture mobility and diffusion in fresh tobacco leaves during drying by the TG–NMR analysis," *J. Therm. Anal. Calorim.*, vol. 135, pp. 2419-2427, 2019, doi: 10.1007/s10973-018-7312-x.

[5] J. Tao *et al.*, "Fermentation process of tobacco leaves drives the specific changes of microbial community," *BMC Microbiology*, vol. 24, no. 1. p. 534, 2024. doi: 10.1186/s12866-024-03702-w.

[6] W. Hu *et al.*, "Fermentation of cigar tobacco leaves with citrus flavonoids: changes in chemical, microbiological, and sensory properties," *Front. Bioeng. Biotechnol.*, vol. 12, no. 1469532, pp. 1-13, 2024, doi: 10.3389/fbioe.2024.1469532.

[7] E. Nurnasari and Subiyakto, "Chemical compound of essential oils from three different area of tobacco leaves (*Nicotiana tabaccum* L.) in Indonesia," *J. Life Sci. Biomed.*, vol. 5, no. 6, pp. 163-166, 2015.

[8] J. Puripattanavong, C. Songkram, L. Lomlim, and T. Amnuaikit, "Development of concentrated emulsion containing nicotiana tabacum extract for use as pesticide," *J. Appl. Pharm. Sci.*, vol. 3, no. 11, pp. 16-21, 2013, doi: 10.7324/JAPS.2013.31104.

[9] M. Mahardika, N. T. Susparini, I. Lailatusholihah, and I. Syahrial, "Ekstrak daun tembakau (*nicotiana tabacum*) sebagai inhibitor korosi alami baja karbon," *J. Inov. Pendidik. dan Sains*, vol. 5, no. 3, pp. 82-87, 2024.

[10] D. Kotzias and O. Geiss, *Tobacco, Cigarettes and Cigarette Smoke*. European Communities, 2007. [Online]. Available: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC37472/7472 - EUR22783EN_Geiss_Kotzias.pdf

[11] V. Popova, Y. Tumbarski, T. Ivanova, R. Hadjikinova, and A. Stoyanova, "Tobacco resinoid (*Nicotiana tabacum* L.) as an active ingredient of cosmetic gels," *J. Appl. Pharm. Sci.*, vol. 9, no. 09, pp. 111-118, 2019, doi: 10.7324/JAPS.2019.90916.

[12] V. Popova, V. Gochev, T. Girova, I. Iliev, T. Ivanova, and A. Stoyanova, "Extraction Products from Tobacco - Aroma and Bioactive Compounds and Activities," *Curr. Bioact. Compd.*, vol. 11, no. 1, pp. 31-37, 2015, doi: 10.2174/1573407211011508041500016.

[13] A. Mindaryani and S. S. Rahayu, "Essential oil from extraction and steam distillation of *ocimum basillicum*," in *Proceedings of the World Congress on Engineering and Computer Science*, 2007, pp. 1-5.

[14] Sugiarto and N. Hamidi, "The role of pre-destilation fermentation in increasing patchouli oil productivity (Pogostemon cablin benth)," in *IOP Conference Series: Materials Science and Engineering*, 2019, pp. 1-10.

[15] S. Changi, T. M. Brown, and P. E. Savage, "Reaction kinetics and pathways for phytol in high-temperature water," *Chem. Eng. J.*, vol. 189-190, pp. 336-345, 2012, doi: 10.1016/j.cej.2012.02.021.

[16] R. L. Rowland, "Flue-Cured Tobacco. II. Neophytadiene," *J. Am. Chem. Soc.*, vol. 79, no. 18, pp. 5007-5010, 1957.

[17] M. Banožić, K. Aladić, I. Jerković, and S. Jokić, "Volatile organic compounds of tobacco leaves versus waste (scrap, dust, and midrib): extraction and optimization," *J. Sci. Food Agric.*, vol. 101, no. 5, pp. 1822-1832, 2021, doi: 10.1002/jsfa.10796.

[18] E. Nurnasari and Subiyakto, "Komposisi kimia minyak atsiri pada beberapa tipe daun tembakau (*Nicotiana tabaccum* L.)," *Ber. Biol.*, vol. 10, no. 5, pp. 571-580, 2011.

[19] R. R. Johnson and J. A. Nicholson, "The structure, chemistry, and synthesis of solanone. a new anomalous terpenoid ketone from tobacco," *J. Org. Chem.*, vol. 30, no. 9, pp. 2918-2921, 1965.

[20] L. C. Cook and A. Rodgman, "The composition of cigarette smoke. XIV. Hexahydrof arnesyl Acetone (Phytone) and 2-Methyl-5-lsopropyl1,3-Nonadien-8-one (Solanone) from turkish tobacco smoke," *Tob. Sci.*, vol. 8, no. 40, pp. 161-162, 1964.

[21] K. Sin-II, Y.-I. Oh, and I. Heu, "Analysis of volatile essential oil playing key role in tobacco leaves," *J. Korean Soc. Tob. Sci.*, vol. 5, no. 2, pp. 47-54, 1983.

[22] A. N. Amin, R. . Long, and W. Weeks, "Transformations of chemical constituents during flue-curing of *Nicotiana tabacum* L. 2. Metabolism of Nitrogenous and Related Constituents," *J. Agric. Food Chem.*, vol. 28, pp. 656-660, 1980, doi: 10.1021/jf60229a024.

[23] M. Zhang *et al.*, "Integrated characterization of fller tobacco leaves: HS–SPME–GC–MS, E-nose, and microbiome analysis across diferent origins," *Bioresour. Bioprocess.*, vol. 11, no. 11, pp. 1-18, 2024.

[24] P. Andrade *et al.*, "Nerolidol production in agroinfiltrated tobacco: Impact of protein stability and membrane targeting of strawberry (*Fragraria ananassa*) nerolidol

synthase1," *Plant Sci.*, vol. 267, pp. 112-123, 2018, doi: 10.1016/j.plantsci.2017.11.013.

[25] L. Zou *et al.*, "Analyzing the effect of microbial consortia fermentation on the quality of HnB by untargeted metabolomics," *J. Microbiol. Biotechnol.*, vol. 34, no. 9, pp. 1890-1897, 2024, doi: 10.4014/jmb.2402.02039.

[26] Y. Meng *et al.*, "Analysis of the relationship Analysis of the relationship between color and natural pigments of tobacco leaves during curing," *Sci. Rep.*, vol. 14, no. 166, pp. 1-10, 2024.

[27] M. L. Gonzalez-Rivera, J. C. Barragan-Galvez, D. Gasca-Martínez, S. Hidalgo-Figueroa, M. Isiordia-Espinoza, and A. J. Alonso-Castro, "In vivo neuropharmacological effects of neophytadiene," *Molecules*, vol. 28, no. 8, p. 3457, 2023.

[28] K. Bhargava, R. Nath, P. K. Seth, K. K. Pant, and R. K. Dixit, "Molecular Docking studies of D2 Dopamine receptor with the Risperidone derivatives," *Bioinformation*, vol. 10, no. 1, pp. 008-012, 2014.

[29] B. J. Oso and I. . Olaoye, "Comparative in vitro studies of antiglycemic potentials and molecular docking of *Ageratum conyzoides* L. and *Phyllanthus amarus* L. methanolic extracts," *SN Appl. Sci.*, vol. 2, no. 629, pp. 1-13, 2020, doi: 10.1007/s42452-020-2275-5.

[30] A. H. Selmy, M. M. Hegazy, A. A. El-Hela, A. M., Saleh, and M. M. El-Hamouly, "In Vitro and In Silico studies of Neophytadiene: A Diterpene Isolated from Aeschynomene Elaphroxylon (Guill. & Perr.) Taub. as Apoptotic," *Egypt. J. Chem.*, vol. 66, no. 10, pp. 149-161, 2023, doi: 10.21608/EJCHEM.2023.178261.7296.

[31] B. U. Maheswari and G. Kalaiselvi, "Exploring neophytadiene from ampelocissus araneosa: a molecular docking approach to inhibit biofilm formation in *Staphylococcus aureus*," *Uttar Pradesh J. Zool.*, vol. 45, no. 3, pp. 59-70, 2024, doi: 10.56557/upjoz/2024/v45i33875.

[32] A. Amutha, T. Jeyalalitha, and P. Muthukumar, "Molecular docking studies of phytoconstituents extracted from the Calotropis gigantea and Strobilanthes kunthiana plants against the target protein carboxylic ester hydrolase from Culex quinquefasciatus and drug design strategies," *Int. J. Entomol. Res.*, vol. 7, no. 9, pp. 75-86, 2022.