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# Chemical Composition and Anti-Microbial Activity of Hog Plum (*Spondias mombin* L.) Peel Oil Extracted from Different Regions of Tropical Climates

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**Abstract:** The hydro-distilled essential oil of hog plum peel may be used for enhancing the flavor and taste of food products as well as for hiding the unpleasant odor of drugs. Thus, the waste peels of *Spondias mombin* appear to have economic importance. To find out the chemical composition and anti-microbial properties of hog plum peel oil, the samples were collected from different regions of Bangladesh for extraction and identification of volatile compounds by GC-MS, where dichloromethane was used as an extraction solvent. The required standard analytical methods were used to assay the anti-microbial properties of hog plums. In this study, pentenyl-3-thy-met-4-alpha-methyl-alpha-ethanol-oxiranen (29.04%), (3,3.1,1)-4-dimethylethyl-1,1-phenol (8.00%), cycohexanol-3 (10.85%), 4-hydroxy-penzeneethanamine (7.09%), hydroxylamine (4.63%), dibutyl phthalate (6.85%), etc., were majorly determined. Consequently, the highest content of 75.81% volatile compounds was found in the Dinajpur district, where the lowest content of 35.00% was found in the Rajshahi district. In contrast, 33 volatile compounds were identified in hog plum peels collected from the Barishal district, whereas 22 compounds were detected in the peel samples collected from the Dinajpur district. In addition, the antimicrobial activity of the oil was analyzed by the disk diffusion method, and the results revealed that the highest Ciprocin content was recorded in the hog plums of Barishal (22.0–23.0 mm), while the lowest was recorded in the Mymensingh sample (20.67–21.63 mm), which was on par with Rajshahi sample (20.70–21.50 mm). The results of the anti-fungal activities of the peel oil showed the highest zone of inhibition against the *Aspergillus niger* (11.63 ± 0.0003 mm) and *Penicillium oxalicum* (13.67 ± 1.97 mm) content of the Rajshahi and Pabna district samples, respectively.

**Keywords:** waste peel; essential oil; GC-MS; volatile compounds; antimicrobial activity; chemical composition



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## 1. Introduction

Hog plum (*Spondias mombin* L.) is a member of the Anacardiaceae family and is locally known as "Amra" in Bangladesh. Its fruit is a drupe characterized with a mixed taste of sour and sweet, and has gained increased importance in modern medicine for its possible pharmacological activities [1,2]. It grows mostly in the Indian subcontinent, e.g., in Bangladesh, India (Assam and Bombay), and Nepal. In Bangladesh, the cultivatable area of hog plum was about 1247 acres, and the corresponding total production was 36,068 metric tons (MT) in 2016–2017 [3,4]. Hog plum is mainly consumed as fresh fruit and has other uses such as making jam, jelly, squash, and marmalades, on a small scale and on a commercial scale. After the consumption and manufacturing of the above products, the peels are discarded as waste that holds almost 20% of the fresh fruit [5,6]. These wastages are considered a problem for the food processing industry and pollution monitoring organizations [7]. Nevertheless, these waste portions could be used as a potential source of valuable by-products like essential oils (EOs) which could be extracted from flowers, leaves, stems, roots, seeds, barks, resins, or fruit rinds [6].

Essential oils from citrus fruits are a group of natural flavors and fragrances which are popularly used in the food and pharmaceutical industries, daily chemical products, and in the health care field [8–12]. The products of medicinal plants like EOs and their antimicrobial properties have been empirically recognized for centuries, but recently the antimicrobial properties have been studied and confirmed scientifically [13–15]. These EOs effectively control the growth of different microorganisms like fungus, yeasts, bacteria, etc., which has been reported in several studies [16–21]. The bark extract of hog plums exhibits a valuable antibacterial activity, while the aqueous extract of the bark has shown a moderate antibacterial activity against *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio cholerae* [22,23].

Many studies have been carried out regarding the fruit, bark, and leaf extracts of hog plums [24–28], but there has been little research work on hog plum peels, which may contain various bioactive volatile and antimicrobial compounds. The extraction and identification of bioactive volatile compounds (VCs) in hog plum peels and their preservation are a potential source of medicine as well as nutrients for functional foods and feed industries.

Therefore, the present study was conducted to discover the chemical composition and anti-microbial properties of hog plum peel oil collected from different regions of Bangladesh.

## 2. Materials and Methods

### *2.1. Sample Collection*

The samples of *Spondias mombin* were collected in the early summer season from the local market of the Dinajpur (Latitude: 25.6221° N, Longitude: 88.6438° E, and Altitude: 42.0 m), Mymensingh (Latitude: 24.7471° N, Longitude: 90.4203° E, and Altitude: 19.0 m), Barishal (Latitude: 22.7010° N, Longitude: 90.3535° E, and Altitude: 1.22 m), Rajshahi (Latitude: 24.3745° N, Longitude: 88.6042° E, and Altitude: 18.0 m), and Pabna districts (Latitude: 24.0023° N, Longitude: 89.1413° E, and Altitude: 19.0 m) of Bangladesh. Three separate samples were collected from each district, which were treated as three replications. The microorganism species used in this study were bacteria (ATCC) such as *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and fungi such as *Aspergillus niger* and *Penicillium oxalicum*. The pure cultures of these species were obtained from the Laboratory of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh. The cultures of each microorganism were inoculated on nutrient agar (NA) and Sabouraud dextrose agar (SDA) (Merck KGaA, 64271 Darmstadt, Germany) at 37 °C for 24 h, and stored at less than 4 °C.

### 2.2. Preparation of Hog Plum Peel Powder

The samples were washed and rinsed with deionized water and subsequently peeled with a knife carefully. The peels of each of the five samples were weighted and spread in five different trays for drying in a cabinet dryer (Model FMA-275) at 60 °C for three days until they were completely dehydrated. Then the samples were taken out from the drier and put into desiccators for a few minutes to adjust to the ambient temperature. Then, the dried samples were blended by an electric blade blender (Vitamix 5200 Series) to make the powdery form and to prepare the peel samples for hydro-distillation.

### 2.3. Extraction of Essential Oil

Five samples of hog plum peels were collected in mid-June 2018 from the five districts. Each sample of peel powder, weighing 20 g, was suspended in 300 mL of deionized water and subjected to steam distillation using a Clevenger-type apparatus for 4 h. Then the sample oils were collected, dried over anhydrous sodium sulfate ($Na_2SO_4$), filtered, and stored in sealed vials under refrigeration at 4°C until analysis [29]. The yield (%) of oil was calculated by the following formula:

$$\text{Yield (\%)} = (\text{Weight of oil})/(\text{Weight of the fresh sample}) \times 100$$

### 2.4. Gas Chromatography and Mass Spectrometry (GC-MS) Analysis

GC-MS was conducted with a Varian Saturn 2200 equipped with an ion trap detector (ITD) for the identification of different components of essential oil. To obtain better results, dichloromethane was used as solvent. The sample of 2.0 µL was injected on a DB-5 MS (30 m, 0.25 mm ID, 0.25 µm film thickness) column. Helium was used as a carrier gas with a flow rate of 1 mL/min and a split ratio of 1:5. The temperature in the oven-dryer was set at 50 °C for 1 min, followed by a temperature gradient of 2.5 °C/min to 280 °C/min for 40 min. The injector and transfer line temperatures were set to 250 °C and 280 °C, respectively. Various components were identified by their retention time (5.52–22.34 min) and peak enhancement with standard samples in gas chromatographic mode and a National Institute of Standards and Technology (NIST 20) library search from the derived mass fragmentation pattern of various components of the essential oil [30].

### 2.5. Determination of Antimicrobial Activity

The antimicrobial activity of the tested essential oil was monitored using the disc diffusion method [31] against different food-borne pathogens including bacteria (*Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*), and two selected fungi (*A. niger* and *P. oxalicum*). The antibacterial and antifungal screening was performed briefly using ciprofloxacin (10 µg/disc) and fluconazole (10 µg/disc) as a positive control and sterile water as a negative control. Standard culture media of each type of bacteria and fungi were employed on NA and SDA plates (100 mL each), where 5 µL (1000 ppm) of the essential oil was used for each test sample. During the investigation, the incubation temperature was maintained for both fungi (25 °C) and bacteria (37 °C). The zones of inhibition thus developed against the tested microorganisms were measured after a period of 48 and 96 h. All experiments were conducted in triplicate. The results of the antimicrobial activity of the peel oil against the different microorganisms were expressed as resistant, intermediate, and sensitive.

### 2.6. Statistical Analysis

Antibacterial and antifungal experiments were performed in triplicate and the analyzed data were presented as mean ± SE. The obtained data were subjected to one-way ANOVA using MS Office 2007 (significance: $p \leq 0.05$; coefficient interval (CV): >95%).

## 3. Results and Discussion

### *3.1. Yield*

The yield of essential oil by the hydro-distillation method was satisfactory from the *Spondias mombin* peel samples collected from the five selected districts of Bangladesh (Table 1). However, the Barishal sample showed the highest production of EOs (75%), while the Pabna sample showed the lowest value (47%). Based on the yield of essential oil, the samples can be ranked as Barishal > Dinajpur > Mymensingh > Rajshahi > Pabna. Natural plant-derived non-phytotoxic substances such as EOs may increase the shelf-life of processed food products by destroying the cell wall of bacteria and fungi. Thus, researchers have devoted their interests to producing natural medicinal and value-added food products from plant-based extracts. The experimental volatile oils of hog plum peels exhibited strong flavor alike to that of the fresh raw samples. Table 1 shows a higher yield of oil from the peels than that obtained from the fruits, leaves, and barks of hog plums [32]. On the other hand, *Mangifera indica* is taxonomically close to *S. mombin* which also had similar results [33,34]. The yield was comparable to that reported in previous studies. Oven-dried citrus peels exhibited higher oil yield followed by the ambient-dried and fresh samples. Soumaya et al. [35] also reported that the yield of volatile compounds varied during ripening and reached the maximum values during the middle stage of maturity (second stage) for citrus fruits, while the highest lemon yield was determined at the beginning of fruit maturation and decreased thereafter.

**Table 1.** Regional samples, oil mass, and its product percentages.

| Regional Sample | Peel Mass (g) | Oil Mass (g) | Yield (%) |
|---|---|---|---|
| Barishal | 20 | 0.15 | 75 |
| Dinajpur | 20 | 0.14 | 70 |
| Mymensingh | 20 | 0.136 | 68 |
| Rajshahi | 20 | 0.118 | 59 |
| Pabna | 20 | 0.094 | 47 |

### *3.2. Chemical Composition of Volatile Oil*

There were 95 chemical compounds in the collected samples of hog plum peels (Table 2), and among them, the esters, alcohols, ketones, aldehydes, acids, hydrocarbons, phenols, and others comprised 26 (27.37%), 23 (24.21%), 16 (16.84%), 9 (9.47%), 6 (6.32%), 4 (4.21%), 5 (5.26%), and 6 (6.32%) of these compounds, respectively. Among the total (95) volatile compounds (VCs) of the five light yellowish oil samples, the Barishal sample oil was found to be rich in pentenyl-3-thy-met-4-alpha-methyl-alpha-ethanol-oxiranen (29.04%), and 5-heptone-methyl-one, 6-2 ester (3.24%); the Dinajpur sample oil contained pentenyl-3-thy-met-4-alpha-methyl-alpha-ethanol-xiranen (21.57%), cycohexanol-3 (8.59), picolylamine (10.49%), (3,3.1,1)-4-dimethylethyl-1,1-phenol (8.00%), 4-hydroxy-penzeneethanamine (7.09%), and dibutyl phthalate (4.32%); the Mymensingh sample oil contained pentenyl-3-thy-met-4-alpha-methyl-alpha-ethanol-oxiranen (28.52%), cycohexanol-3 (10.85%), dibutyl phthalate (6.85%), and cyclotetrasiloxane (1.94%); the Rajshahi sample oil contained pentenyl-3-thy-met-4-alpha-methyl-alpha-ethanol-oxiranen (9.27%), cycohexanol-3 (5.80%), borneol (2.35%), and hydroxylamine(4.63%); and the Pabna sample oil was found to be rich in pentenyl-3-thy-met-4-alpha-methyl-alpha-ethanol-oxiranen (11.45%), cycohexanol-3 (25.00%), borneol (4.96%), dibutyl phthalate (3.03%), and 3-cyclohexen-1-ol (3.57%). Furthermore, pentenyl-3-thy-met-4-alpha-methyl-alpha-ethanol-oxiranen was detected in all the samples, but cycohexanol-3 was present only in the Mymensingh, Dinajpur, Rajshahi, and Pabna samples, and was absent in the Barishal samples. (Methylyethyl-1)-4-methyl-oxabicyclo, 1-7 was present in the Dinajpur, Barishal, and Pabna samples but was absent in the Rajshahi and Mymensingh samples. Cycohexanol-3 was present in the Mymensingh, Rajshahi, Dinajpur, and Pabna samples but was absent in the Barishal sample. Dibutyl phthalate was present in the Dinajpur,

Pabna, and Mymensingh samples, but was absent in the Barishal and Rajshahi samples, and so on.

**Table 2.** Chemical compounds obtained from hog plum samples by GC-MS (Retention time: 5.52–22.34 min).

| Sl. No. | Chemical Compound | Amounts (%) | | | | |
|---|---|---|---|---|---|---|
| | | Barishal | Dinajpur | Mymensingh | Rajshahi | Pabna |
| 1 | Propen 1, 2, 3 trichloro benzene | 0.24 | - | - | - | - |
| 2 | Epoxycarane-3, 2 | 0.85 | - | - | - | - |
| 3 | Chloride-butyl-benzene, tert | 0.47 | - | - | - | - |
| 4 | Pentenyl-3-thy-met-4-alpha-methyl-alpha-ethanol-oxiranen | 29.04 | 21.57 | 28.52 | 9.27 | 11.45 |
| 5 | Acetate, -benzenethanol, alpha, dimethyl | 0.14 | - | - | - | - |
| 6 | Methyl-nonyne, 7-1 | 0.12 | - | - | - | - |
| 7 | (Fenchol)-trimethyl-ol, 1, 3, 3-2-bichlo(2.2.1)hepton | 0.17 | - | - | - | - |
| 8 | (Methylethyl-1)-4-methyl-oxabicyclo(4.1.6) hepton, 1-7- | 1.37 | - | - | - | - |
| 9 | Nonennal, (E)-6 | 0.64 | - | - | - | - |
| 10 | (Methylyethyl-1)-4-methyl-oxabicyclo, 1-7 | 1.37 | 0.56 | - | - | 2.13 |
| 11 | 3,1-Cycloheptadiene | 0.44 | - | - | - | - |
| 12 | (Yl-buten 3-1)-bicyclo (2.2.1) hepton, 2 | 1.44 | - | - | - | - |
| 13 | 10-Methyl-8-ol acetate-1-tetradecen | 0.49 | - | - | - | - |
| 14 | 4,1-Cyclohexadiene-(methyl-1)-methanol,4-1 | 0.49 | - | - | - | 0.39 |
| 15 | Exo-2-hydroxycineole | 0.36 | - | - | - | - |
| 16 | 5-Heptone-methyl-one, 6-2 ester | 3.24 | - | - | - | - |
| 17 | (Ethyl-methyl-1)-benzaldehyde,4 | 0.28 | - | - | 0.04 | 0.01 |
| 18 | Methyl-bromo-ene,7-7-pentadec | 0.26 | - | - | - | - |
| 19 | (Ethyl-methyl-1)-1-methyl-ene,4-2-bicyclo(3.1.0)hexan- | 0.92 | - | - | - | - |
| 20 | (Ethyl-methyl-1)-5-methyl-phenol,2 | 0.73 | 0.29 | 0.14 | 0.04 | 0.31 |
| 21 | Terpenyl acetate-alpha-epoxy | 0.83 | - | 0.12 | 1.36 | 0.61 |
| 22 | Hylidenne-methanol,2-6, bicyclo (3.1.0) hexane | 0.53 | - | - | - | - |
| 23 | 9-E-8-Methyl-ol, acetate-2-tridecel | 0.71 | - | - | - | - |
| 24 | 15,12,9-bis (a)-octadecatrienoicacid, 2,3 | 0.09 | - | - | - | - |
| 25 | Diene, 2-[8, (7) 1]-menpha-p-2r,4r-hydroperoxide | 0.58 | - | - | - | - |
| 26 | 8-Hydroxycarvctancetone | 0.08 | - | - | - | - |
| 27 | 2,5-Dihydro-3,4-furanacetic acid | 0.38 | - | - | - | - |
| 28 | 3-Buten-2-one-4[2,6,6-trimethyl-1-cyclohexen] | 0.11 | - | - | - | - |
| 29 | 2-(3,4-Dibromo-4-methyl cyclohexyl) propanol | 0.20 | - | - | - | - |
| 30 | Phenol, 2,4-bis (1.1 dimethylehyl) | 0.06 | 0.68 | 0.46 | - | 0.30 |
| 31 | Nonadecane | 0.11 | - | - | 0.65 | - |
| 32 | Hydroxylamine-O-decylamine | 0.09 | - | - | - | - |
| 33 | Phthalic acid, isobutylnonyl ester | 0.96 | - | 0.45 | 0.46 | - |
| 34 | Cycohexanol-3 | - | 8.59 | 10.85 | 5.80 | 25.00 |
| 35 | Cycohexanol, 5-methyl-2(1-methylethen) | - | 0.80 | - | - | - |

**Table 2.** *Cont.*

| Sl. No. | Chemical Compound | Amounts (%) | | | | |
|---|---|---|---|---|---|---|
| | | **Barishal** | **Dinajpur** | **Mymensingh** | **Rajshahi** | **Pabna** |
| 36 | Isopulegol acetate | - | 0.50 | - | - | 1.91 |
| 37 | Borneol | - | 0.82 | - | 2.35 | 4.96 |
| 38 | 3-Acetoxy-p-menthane-3-one | - | 0.41 | - | - | - |
| 39 | 2-Methyl-3-(1-methylethyl cyclohexanol) | - | 0.18 | - | 0.89 | - |
| 40 | Carbamic acid, N-(1,1-ethyl bistrifluoron) | - | 0.82 | - | - | - |
| 41 | (3,3.1, 1)-4-Dimethylethyl-1,1-phenol | - | 8.00 | - | - | - |
| 42 | Picolyamine | - | 10.49 | - | - | - |
| 43 | 3,4-Methyl-dimethyl 2,3-butyryl benzoate | - | 0.57 | - | - | - |
| 44 | Phthalicacid, 2-acetylphenyl heptyl ester | - | 0.44 | - | - | - |
| 45 | 2-Phenylquinazolin-4-ol | - | 1.38 | - | - | - |
| 46 | Dibutyl phthalate | - | 4.32 | 6.85 | - | 3.03 |
| 47 | 4-Hydroxy-penzeneethanamine | - | 7.09 | - | - | - |
| 48 | 2,4-Dimethyl-5,6,11,12 tetraaza | - | 2.71 | - | - | - |
| 49 | 4-Oxo-1,2,3,4,7,12-octahydropy- | - | 1.46 | - | - | - |
| 50 | 2,2-(Dimethyl-1,1-bisene-6-methylethyl-phenol | - | 3.84 | - | - | - |
| 51 | 4-(1-methylethyl) benzenmethanol | - | 0.29 | 0.29 | - | - |
| 52 | Cyclotetrasiloxane | - | - | 1.94 | - | - |
| 53 | Benzene, 1-methyl-4(1-methylethenyl) | - | - | 0.07 | - | - |
| 54 | 1,7-Octaden-3-ol, 2,6-dimethylamin | - | - | 0.07 | - | - |
| 55 | Decamethylcyclopentasiloxane | - | - | 0.31 | - | - |
| 56 | 1,3,3-Trimethylbicyclo[2.2.1]-heptan-2-ol propanoate | - | - | 0.47 | - | - |
| 57 | 3-Cyclohexen-1-ol | - | - | 0.32 | 0.73 | 3.57 |
| 58 | Isopuleggol acetate | - | - | 1.87 | 0.24 | 1.22 |
| 59 | Borneal | - | - | 0.78 | - | - |
| 60 | Bicyclo (3.1.0) hexan 4-methyl-1 | - | - | 1.30 | - | - |
| 61 | P-menth-2-en-7-ol, trans | - | - | 0.81 | - | - |
| 62 | Nonynoic acid, 7-methyl ester | - | - | 0.34 | - | - |
| 63 | 5,7-Dodecadiyl, 1,12-diol | - | - | 0.32 | - | - |
| 64 | O-decyl-hyroxylamine | - | - | 0.21 | - | - |
| 65 | Hyroxylamine, O-decyl- | - | - | 0.28 | - | - |
| 66 | Acetic acid, Chloro-albydrate | - | - | 0.24 | - | - |
| 67 | (E)-2-Octenal | - | - | - | 0.03 | - |
| 68 | 7-methyl-1-Nonyne | - | - | - | 3.52 | - |
| 69 | Exo-Fenchol | - | - | - | 0.11 | - |
| 70 | 2,4-Pentadien-1-ol,3-pentyl-, 2Z | - | - | - | 0.73 | - |
| 71 | Isobornyl formate | - | - | - | 0.15 | - |
| 72 | Sanitolina alcohol | - | - | - | 0.78 | - |
| 73 | 1,3,3,-Trimethyl-2-oxabicyclo[2,2,2] octan-6-ol | - | - | - | 0.18 | - |
| 74 | 2-Octen-1-ol, 3,7 –dimethyl –isobutyrate | - | - | - | 0.12 | 0.06 |
| 75 | (E)-8-Methyaltetradec 1-ol acetate | - | - | - | 0.37 | - |

**Table 2.** *Cont.*

| Sl. No. | Chemical Compound | Amounts (%) | | | | |
|---|---|---|---|---|---|---|
| | | **Barishal** | **Dinajpur** | **Mymensingh** | **Rajshahi** | **Pabna** |
| 76 | 10 Methyl-8-tetradecen-1-ol acetate | - | - | - | 1.69 | - |
| 77 | Phthalic acid, 4-butyl-octyl ester | - | - | - | 0.69 | - |
| 78 | Hydroxylamine | - | - | - | 4.63 | - |
| 79 | 2-Methyl-2-propenyl benzene | - | - | - | 0.17 | - |
| 80 | 1-Pentadecyne | - | - | - | - | 0.56 |
| 81 | Bicyclo (1,2,2) heptan-2-ol, 1,3,3 trimewthyl- | - | - | - | - | 0.53 |
| 82 | (E)—6 Nonenal | - | - | - | - | 0.77 |
| 83 | Beta-cisterpineol | - | - | - | - | 0.56 |
| 84 | Trifluoro-epiiso-bomeol | - | - | - | - | 0.21 |
| 85 | Santolina alcohol | - | - | - | - | 1.46 |
| 86 | 1,5,5-Trimethyl—6-methylene cyclohexen | - | - | - | - | 0.30 |
| 87 | E-3 Bicyclo[2.1.1] trihepten | - | - | - | - | 0.91 |
| 88 | Trans-m-2,8-mentha –dienol | - | - | - | - | 0.22 |
| 89 | Bicyclo [3.1.0] hexane 2-one 5,5,6-trimethyl- | - | - | - | - | 0.96 |
| 90 | Bicyclo [3.1.0] hexane, 6-(1-isopropylidene) | - | - | - | - | 1.19 |
| 91 | 1-Methyl-3-(1-methylethenyl) cyclohexane | - | - | - | - | 0.67 |
| 92 | Eugenol | - | - | - | - | 0.92 |
| 93 | 2,4,4-Trimethyl-1,5-dienyl cyclohexane | - | - | - | - | 0.16 |
| 94 | 4-Hexyl 2,5-dihydro-3-acetic acid | - | - | - | - | 0.15 |
| 95 | 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester | - | - | - | - | 0.78 |
| | Not identified (N.I) | 51.72 | 23.90 | 42.23 | 64.38 | 34.42 |
| | Identified | 47.79 | 75.81 | 57.01 | 35.00 | 65.30 |
| | **Total (%)** | **99.51** | **99.23** | **99.24** | **99.38** | **99.72** |

Significant spatial variations were observed in the number of VCs detected in the hog plum peel samples (Figures 1–3). The number of VCs varied among hog plum peel samples (Figure 1 and Table 2). The highest number of VCs (75.81%) was recorded in samples collected from Dinajpur followed by those collected from Pabna (65.30%), Mymensingh (57.01%), and Barishal (47.79%), while the lowest number of VCs (35.00%) was observed in the samples collected from Rajshahi. Figure 2 represents the number of VCs, which were identified by GC-MS analysis of *S. mombin* from the five districts of Bangladesh. The numerical values of the chemical compounds of hog plum peel essential oil from Dinajpur, Mymensingh, Barishal, Rajshahi, and Pabna were 22, 23, 33, 24, and 30, respectively. It was reported earlier that the number of VCs present in the essential oils of hog plum peels is comparatively higher than that of mango peels [34]. This is in agreement with the earlier literature studies, which showed a considerable variation in the chemical composition of peel EOs concerning varieties and drying conditions [36]. The investigational components of the oil were higher in number than those of earlier literature which was held on the fresh fruits, leaves, and barks of hog plum cultivated in the tropical conditions of Brazil [37]. Several studies also considered the volatile oil of mango fruits, leaves, and peels with results comparable to those of hog plum peel oil. Each of the volatile oils exhibited different chemical constituents that may be attributed to several factors such as ecological and climatic conditions, plant age, and genotypic characteristics [38–43]. The

esters showed the highest value (27.37%), and hydrocarbons showed the lowest value (4.21%) among the volatile substances in this study. Phthalic acid, isobutyl nonyl ester, and 2-phenylheptyl ester-acetyl-phthalic acid were detected in lower quantities. These substances, produced biosynthetically from unsaturated fatty acids, are precursors for the straight-chain esters [44]. Dichloromethane was used because generally, it appears as the best solvent for extraction of a wide class of flavors. The GC-MS analysis revealed that the hog plum peel oil contained different kinds of chemical compounds and/or EOs (Table 2) which have numerous antimicrobial properties that efficiently regulate the growth of different microorganisms such as fungus, bacteria, and yeasts, as envisaged earlier in several studies [21,22]. It has been proven that these compounds are potentially useful additives for food preservation against mycotoxigenic fungi and bacteria. A similar conclusion was suggested from a recent study, which ascertained that these additives prolong the shelf life and improve the quality of stored food products [45].

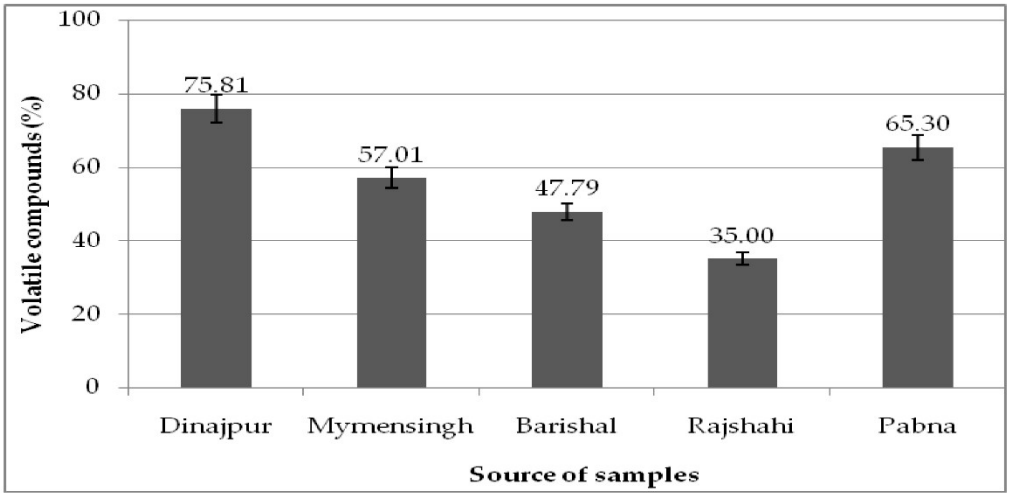


**Figure 1.** Yield of volatile compounds from hog plum peel samples collected from different districts of Bangladesh.

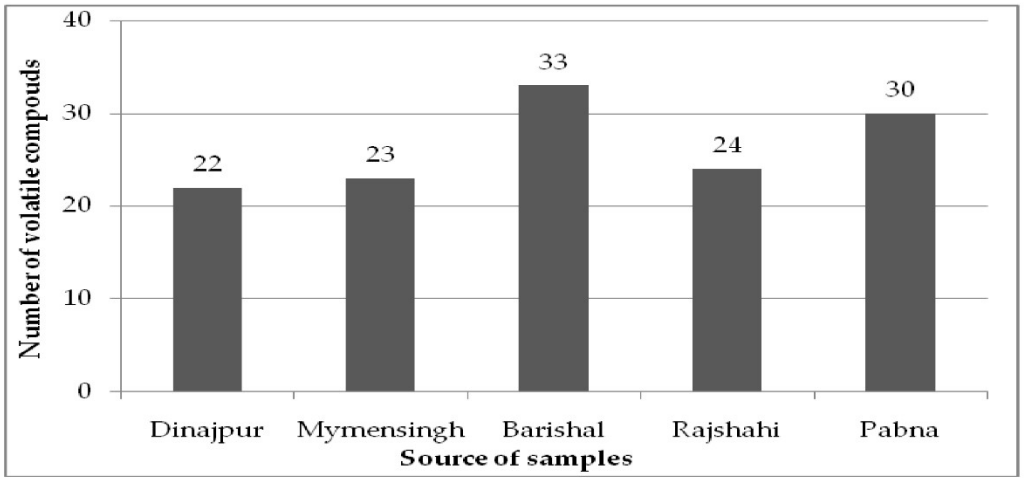


**Figure 2.** Number of volatile compounds identified from hog plum peel samples collected from different districts of Bangladesh.

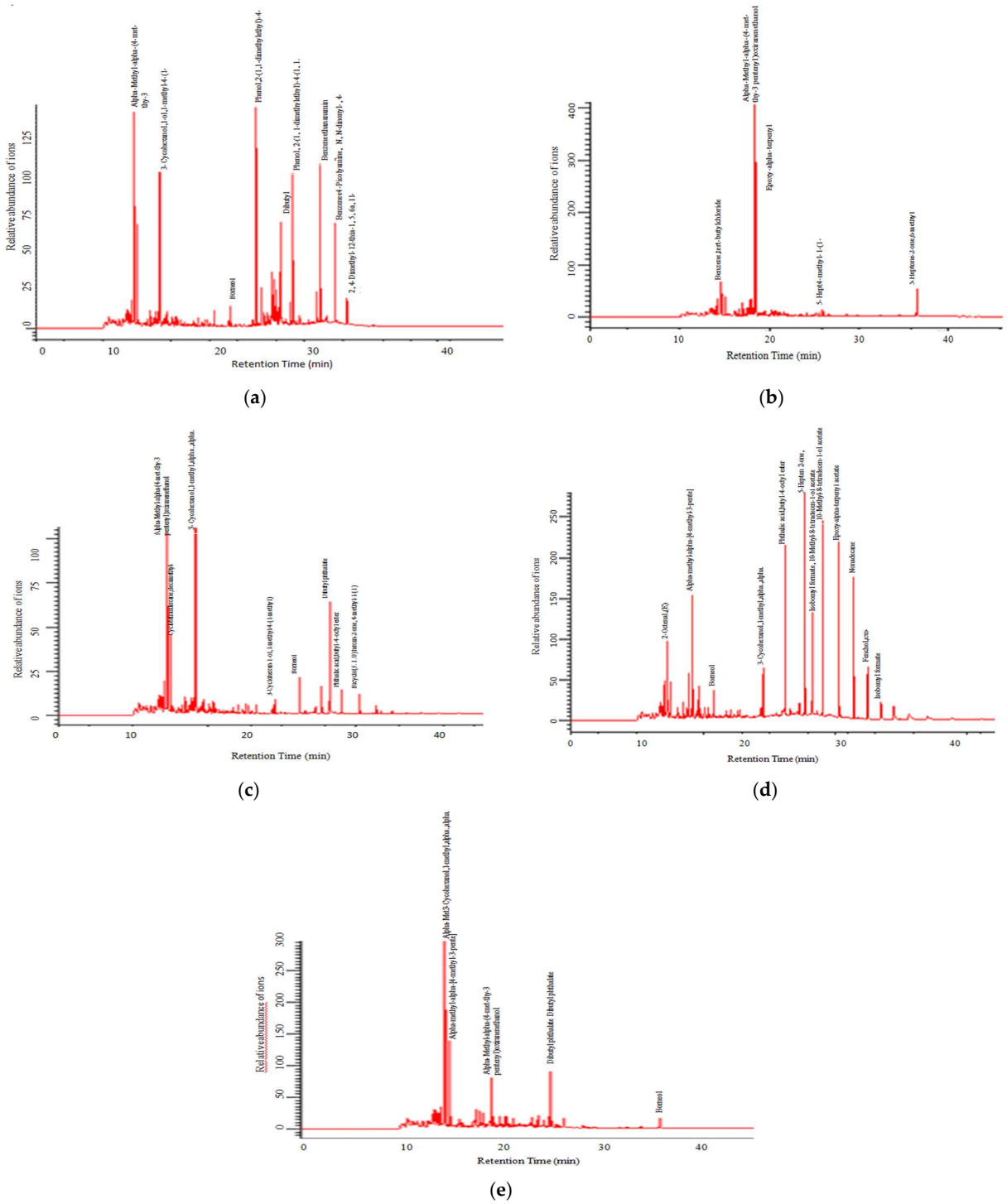


**Figure 3.** GC-MS chromatogram of *S. mombin* peel oil collected from (**a**) Dinajpur, (**b**) Barishal, (**c**) Mymensingh, (**d**) Rajshahi, and (**e**) Pabna districts.

### *3.3. Anti-Bacterial Activities of Essential Oil*

In Table 3, antibacterial activity of peel oil was determined against three bacterial pathogens, namely, *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*. The disk diffusion method was used to determine the antimicrobial activity by measuring the zone of inhibition. All peel oils exhibited a moderate clearance zone of inhibition against *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*. Among those, the Barishal sample oil showed the highest antibacterial sensitivity. From the experimental data, the Barishal

sample oil showed inhibition diameters of 14 ± 0.17 mm, 13 ± 0.29 mm, and 12 ± 0.17 mm for *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp., respectively. However, the standard Ciprocin exhibited anti-bacterial activity ranging from 22 to 23 mm. The obtained oil samples showed significant or moderate antimicrobial and antifungal activity against clinically isolated pathogenic microbial strains in comparison to standard Ciprocin and fluconazole; hence, it might be considered essential to their potential for maintaining hygienic, healthy conditions. These results are similar to those of Chacko and Estherlydia [7]. Ciprocin (Ciprofloxacin) is a fluoroquinolone antibiotic used to prevent bacterial infections such as bone and joint infections, intra-abdominal infections, certain types of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, urinary tract infections, etc. Results from the third disk diffusion test of the peel oil, represented in Table 3, show that the gram-positive *S. aureus* bacteria was more sensitive to the tested extracts than the other two gram-negative bacteria. Most studies have investigated the action of EOs against food spoilage microorganisms and food-borne pathogens and showed that these oils are slightly more active against the gram-positive than the gram-negative bacteria [46]. They studied the resistance against bacteria and represented the inhibition zones against the ciprofloxacin and erythromycin. These results are comparable to those of our research. Kalemba et al. [47] classified the antimicrobial activity of EOs into three levels: weak activity (inhibition zone ≤ 12 mm), moderate activity (12 mm ≤ inhibition zone ≤ 20 mm), and strong activity (inhibition zone ≥ 20 mm), while our samples showed moderate to strong antimicrobial activity (Table 3). Moreover, several researchers have studied the antifungal activities of important *Spondias* species [48].

### *3.4. Anti-Fungal Activity of Essential Oil*

As shown in Table 4, the antifungal activity of the peel oil samples was determined against two fungal pathogens, namely, *A. niger* and *P. oxalicum*. The disk diffusion method was used to determine the bioactivity by measuring the zone of inhibition. From our research data, peel oil from the Pabna sample produced satisfactory inhibition against *A. niger* and *P. oxalicum*. However, the highest inhibition zone of *P. oxalicum* (13.67 ± 1.97 mm) was observed against the Pabna sample oil, whereas *A. niger* showed the best inhibition against the Rajshahi sample oil (11.63 ± 0.0003 mm). The Barishal sample oil showed the second-highest zone of inhibition against *P. oxalicum* (13.17 ± 0.17 mm) followed by *A. niger* (11.33 ± 0.17 mm). On the other hand, standard fluconazole exhibits anti-bacterial activity ranges from 19 to 20 mm. The antifungal results of all the essential oil samples in our study were similar to the results of previous research [49]. The monoterpenes of the volatile oils were mainly terpene hydrocarbons that have antimicrobial activity [50–53]. These monoterpene hydrocarbons inhibit both bacteria and fungi via interference with spore germination and mycelia growth [54–56]. Most of the terpenoids and their derivatives found in this study are important natural medicinal chemical constituents with wide biological activities [33]. The phenolic compounds can donate a hydrogen atom to the free radicals, thus breaking the propagation of chain reactions during the lipid oxidation process [57,58]. The oil can also inhibit the activity of protective enzymes and sequentially inhibit one or more biochemical pathways [11]. Lu et al. [59] explained the effect of oxygen availability on the antimicrobial efficacy of the oil on *Staphylococcus aureus* and *Salmonella enteritidis*. Microaerobic or anaerobic conditions were greatly enhanced when these organisms were incubated. The antimicrobial components of the essential oil cross the cell membrane, interact with the enzymes and proteins of the membrane, and hence produce a flux of protons towards the cell exterior, which induces the changes in the cells and ultimately, promotes their death. Due to the presence of monoterpenes in EOs, they may be used extensively as natural preservatives in many food products, soaps, soft drinks, cosmetics, and perfumes for their lemon-like flavor and odor [48].

**Table 3.** Antibacterial activity of hog plum (*Spondias mombin* L.) peel oil extracted from different regions of Bangladesh.

| SN | Bacterial Strains | Diameter of Zone of Inhibition (mm) * | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BS | | DS | | MS | | RS | | PS | |
| | | Cultures (5 µL/Petridish) | Standard (Ciprocin) (10 µg/disc) | Cultures (5 µL/Petridish) | Standard (Ciprocin) (10 µg/disc) | Cultures (5 µL/Petridish) | Standard (Ciprocin) (10 µg/disc) | Cultures (5 µL/Petridis) | Standard (Ciprocin) (10 µg/disc) | Cultures (5 µL/Petridish) | Standard (Ciprocin) (10 µg/disc) |
| 1 | *Salmonella* spp. | 12.00 ± 0.17 | 23.00 ± 0.58 | 11.50 ± 0.01 | 22.5 ± 0.007 | 10.87 ± 0.015 | 21.63 ± 0.01 | 10.50 ± 0.007 | 21.50 ± 0.007 | 12.80 ± 0.03 | 22.00 ± 0.20 |
| 2 | *Staphylococcus aureus* | 14.00 ± 0.17 | 22.00 ± 0.27 | 13.83 ± 0.04 | 21.96 ± 0.0004 | 13.13 ± 0.001 | 21.00 ± 0.007 | 13.40 ± 0.190 | 21.40 ± 0.009 | 13.90 ± 0.0001 | 21.87 ± 0.05 |
| 3 | *Escherichia coli* | 13.00 ± 0.29 | 22.00 ± 0.22 | 12.7 ± 0.015 | 21.26 ± 0.019 | 12.37 ± 0.01 | 20.67 ± 0.01 | 13.17 ± 0.001 | 20.70 ± 0.02 | 13.40 ± 0.005 | 22.00 ± 0.67 |

* Values are represented as the mean ± S.D. of three experiments; BS = Barishal sample, DS = Dinajpur sample, MS = Mymensingh sample, RS = Rajshahi sample, and PS = Pabna sample.

**Table 4.** Antifungal activity of hog plum (*Spondias mombin* L.) peel oil extracted from different regions of Bangladesh.

| SN | Fungal Strains | Diameter of Zone of Inhibition (mm) * | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BS | | DS | | MS | | RS | | PS | |
| | | Cultures (5 µL/Petridish) | Standard (Fluconozole) (10 µg/disc) | Cultures (5 µL/Petridish) | Standard (Fluconozole) (10 µg/disc) | Cultures (5 µL/Petridish) | Standard (Fluconozole) (10 µg/disc) | Cultures (5 µL/Petridish) | Standard (Fluconozole) (10 µg/disc) | Cultures (5 µL/Petridish) | Standard (Fluconozole) (10 µg/disc) |
| 1 | *A. niger* | 11.33 ± 0.17 | 19.13 ± 0.35 | 11.27 ± 0.02 | 19.43 ± 0.01 | 11.07 ± 0.0002 | 18.90 ± 0.0002 | 11.63 ± 0.0003 | 18.9 ± 0.020 | 11.10 ± 0.0005 | 19.73 ± 0.0005 |
| 2 | *P. oxalicum* | 13.17 ± 0.17 | 20.15 ± 0.77 | 12.93 ± 0.02 | 20.15 ± 0.77 | 12.87 ± 0.0004 | 19.87 ± 0.004 | 12.93 ± 0.002 | 19.3 ± 0.003 | 13.67 ± 1.97 | 20.37 ± 0.001 |

* Values are represented as the mean ± S.D. of three experiments; BS = Barishal sample, DS = Dinajpur sample, MS = Mymensingh sample, RS = Rajshahi sample, and PS = Pabna sample.

## 4. Conclusions

This study showed that the peel of *S. mombin* appears to be unique in terms of its volatile composition. The results indicate that the essential oils of different regions showed varying anti-microbial activities against the various food-borne pathogenic bacteria and fungi tested. The highest zone of inhibition was shown against *Staphylococcus aureus* and *Penicillium oxalicum*. On the other hand, the highest number of VCs was identified in peel samples from the Barishal district, whereas the lowest number was in the Dinajpur district samples. On the contrary, the highest number of VCs was found in the Dinajpur district samples, whereas the lowest number was in the Rajshahi district samples. However, the variation in the number of VCs of the samples was very small. Thus, *S. mombin* could become an alternative to synthetic bactericides and fungicides for use in agro-industries. This study provides data for the development of a more economical, useful, and eco-friendly bio-alternative to existing usages of hog plum peels. However, further studies on the safety and toxicity of these oils and their possible in vivo bioactivity should be carried out before use.

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