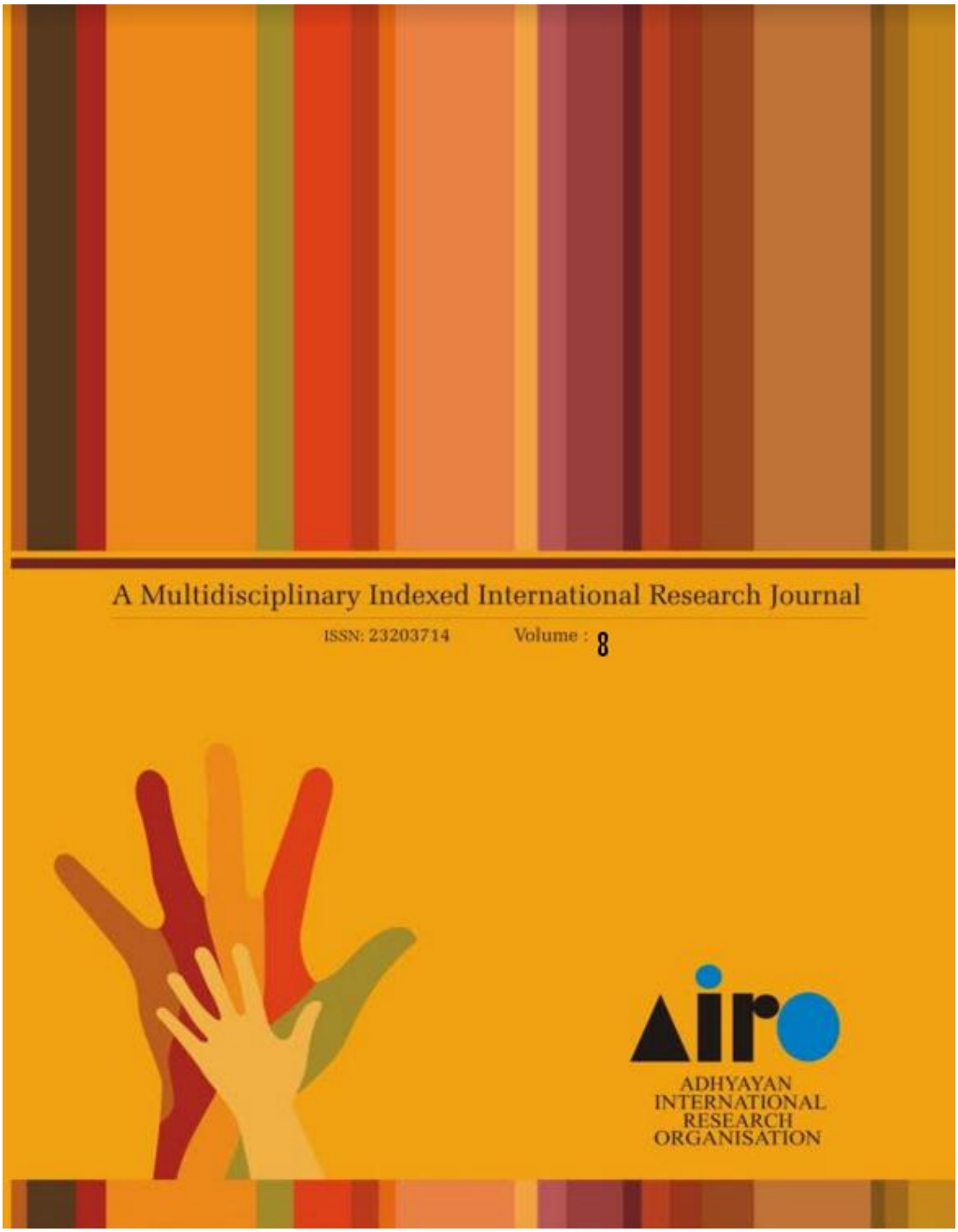
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A STUDY ON PIPER BETLE LEAF AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

The Betel oil from the leaves of Piper betle L obtained through steam distillation was characterized for its organoleptic properties and physicochemical constants. Freshly extracted Betel oil is pale yellow, soluble in common organic solvents and very slightly soluble in water. Its specific gravity is 0.9313; with 1.4526 as refractive index; +4.249 optical activity and ester value of 101.

The chemical components of the oil identified via Gas Chromatography-Mass Spectroscopy consist of 5-(2-propenyl)-1, 3-benzodioxole, eugenol isomer and caryophyllene among others.

KEYWORDS: *Solubility, Properties, Oil*

INTRODUCTION

The minimum effective concentration (MIC) of the oil was determined using Dilution method. The oil was found to have significant antibacterial and antifungal activity against *Staphylococcus aureus, Streptococcus pyogenes, Candida albicans* and *Trichophyton mentagrophytes* with MIC values of: 125 µg/ml; 15.60 µg/ml; 250µg/ml and 195µg/ml respectively. Antimicrobial activity was tested by agar diffusion method utilizing the same test organisms. The zones of growth inhibition measured to the nearest mm. were: 67.50 mm for *S. aureus*; 90 mm for S. *pyogenes, C. albicans* and *T. mentagrophytes.*

It is well known that intensive use of an antibiotic is often followed by the appearance of resistant strains. In view of this, the search for new antimicrobial agents continues unabated. Medicinal plants are promising resources. The use of medicinal plants as screening pool for novel antibiotics has several advantages related to safety, availability, and minimizing the risk of side effects and addiction (Lee et al., 2003). The World Health Organization adopted a major policy change in accepting that most

developing nations would have to make use of more traditional medical practices for primary health care. (Yuan and Lin, 2005).

Piper betle is a glabrous climbing vine belonging to the family Piperaceae. It is abundantly distributed in many Asian countries. The leaves have been used in traditional medicine as carminative, stimulant, antiseptic, antifungal, and antibacterial agent. The volatile oil known as Betel oil is the chief constituent of the leaves. *Piper betle* L. can be of great benefit in treating diseases caused by bacteria and fungi. Previous studies on the betel leaves, roots and whole extract (mixture of volatile and non-volatile) of the green variety showed a very strong antimicrobial activity (Jenie , 2001).

The oil was characterized by determining the physical and chemical properties. Antimicrobial activity was evaluated by the lowest concentration that will inhibit the growth of the test organisms by Dilution method. The zone of growth inhibition caused by the Betel oil in the Agar diffusion test was also measured as an indication of its activity.

An antimicrobial agent is a substance that kills or inhibits the growth of microorganisms. It may be categorized on the basis of their antibacterial activity as either bacteriostatic or bactericidal. Some volatile oils have antifungal activity. These are agents capable of destroying or inhibiting the growth of fungi. These agents may either be fungicidal or fungistatic. The study was conducted to prove that the volatile oil of the vine is the chief constituent that causes its antimicrobial activity.

Materials and Methods

The mature *Piper betle L*. leaves was collected from La Union, Abra, Iloilo City, Palawan and Malaybalay, Bukidnon. A representative of the whole plant was brought to the Philippine National Museum for authentication. The collected leaves were washed, dried between filter chapters and air-dried. The leaves were cut into small pieces for extraction using steam distillation and isolated by rotary evaporation. The percentage yield of the oil was computed on the basis of the air-dried material.

Physical characterization of the *Piper betle* L. volatile oil consists of the description

and determination of attributes. The volatile oil was placed in a transparent bottle over a white background and the color and clarity were observed; the characteristic odor was determined by sniffing; and to determine its characteristic feel to the touch, it was rubbed between fingers.

The solubility of the *Piper betle* volatile oil was determined by mixing increment volumes of the volatile oil in specified volumes of the following solvents: water, chloroform, alcohol, anhydrous ether and petroleum ether**.**

Specific gravity is an important criterion of the quality and purity of volatile oils. The actual weight or the tare of a vial or was determined accurately using a Sartorius CP135 Balance. The vial was filled with water and weighed. The procedure was repeated using the *Piper betle* volatile oil in place of water. The specific gravity of the oil is expressed as the ratio of the weight of the volume of oil to that of an equal volume of pure water when both are determined at 25oC (Knevel and DiGangi, 1977).

Both the degree of rotation and its direction are important criteria of purity. The extent of optical activity of oil was determined by a polarimeter (E. Harnack 220) which measured the degree of rotation. The zero point of the polarimeter was adjusted and determined. The previously cleaned and dried polarimeter tube was filled with 10% alcoholic solution of the volatile oil. The analyzer was rotated until equal illumination of light of the two halves of the visual field is achieved (Knevel and DiGangi, 1977).

Chemical Analysis of the Piper betle Volatile Oil

The *Piper betle* volatile oil was brought to the National Chemistry Instrumentation Center (NCIC) at Ateneo de Manila, Quezon City for its chemical characterization using Gas Chromatography- Mass Spectroscopy**.** Determination of the total esters serves to detect adulteration and to establish the quality and purity of valued oils.

About 1.5 to 2 grams of the oil, accurately weighed was placed in a 250-mL Erlenmeyer flask to which 10 mL of neutralized alcohol and 2 drops of phenolphthalein TS was added drop wise. Then 0.1N sodium hydroxide solution was added until a faint pink color appeared.

After adding 25.0 mL of 0.5N alcoholic potassium hydroxide, a reflux condenser was connected and was then heated on a boiling water bath for 1 hour.

The mixture was allowed to cool, about 20 mL of water and 3 drops of phenolphthalein TS was then added and the excess alkali was titrated with 0.5N hydrochloric acid. A blank test was performed and the total ester was calculated using the following formula (British Pharmacopoeia, 1980):

$$\text{Ester Value} = \frac{(\text{mL of 0.5NHCl} - \text{mL blank}) \times 0.5\text{N HCl} \times 56.11\text{mg/mEq}}{\text{Weight of sample}}$$

Preliminary Screening of the Antimicrobial Activity of Piper betle volatile oil

Results and Discussions

The matured leaves were collected from La Union, Abra, Iloilo, Palawan and Malaybalay. A total of 33.759 kilograms was gathered. Approximate weights are shown as follows:

Place	Approximate Weights (Kg)
Abra	1.200
Iloilo	3.100
La Union	25.250
Malaybalay	3.250
Palawan	*0.959*____
Total	33.759

A representative of the plant sample was authenticated by the Philippine National Museum in Manila.

The air dried leaves were subjected to steam distillation in order to extract the volatile oil. Two hundred fifty six (256) mL was obtained from about 18 kilograms of air dried leaves. The steam distillation process had a standard deviation of 0.0951675, which is equivalent to 6.5946% relative standard deviation (RSD). The % of RSD indicates that experimental method of steam distillation is imprecise due to wastage (specification: precision = maximum of 2%). Table 10 shows an

average percentage yield of 1.4431% of the oil from 5 batches.

Table 1: Percentage yield (v/wt) Volatile Oil in *Piper betle* Leaves.

Batch Number	Wt. of Leaves (Kg)	Volume of Oil	Percentage Yield (%v/w)
1	4.9833	73.00	1.4649
2	1.0248	14.50	1.4149
3	0.6067	9.70	1.5988
4	1.0960	15.00	1.3686
5	10.5258	144.00	1.3681
		Σ = 256.20	Ave =1.4431

Physical Properties and Constants of the Piper betle L. Volatile Oil

Organoleptic evaluation of the volatile oil was done and the following properties were noted: color, odor, taste and feel to the touch. The solubility in different solvents such as water, ethyl alcohol, chloroform, anhydrous ether and petroleum ether were determined. Physical constants such as specific gravity, optical rotation and refractive index of the volatile oil were also determined.

It is immiscible in water in the ratio of 0.1:0.1 but was soluble in 50.0 mL of water or no separation of phase was observed. Most volatile oils are miscible in organic solvents but sufficiently soluble in water but sufficiently soluble to form a saturated solution and impart its odor to the water. The *Piper betle* volatile oil also possesses this property of volatile oils (Tyler, et al., 1988).

Table 2: Solubility of *Piper betle* Volatile Oil

Solvent	Volume Ratio (Oil:solvent) mL	Description
Water	1:1	immiscible
	1:5	No separation of phases but cloudy
Ethyl alcohol	1:1	miscible
Chloroform	1:1	miscible
Anhydrous ether	1:1	miscible
Petroleum ether	1:1	miscible

Physical constants serve as a means of assessing the purity and quality of the volatile oil as well as for identification. The specific gravity, optical activity and refractive index were determined. The table below shows the average values obtained from these determinations**.**

Conclusion

Medicinal plants have played a vital role in treating diseases and in promoting health of mankind for a long time. They continue to become an important source of medicinal agents. The utilization of local sources as alternative drug can be more or equally effective as the synthetic counterpart. The ability to assure the physical and chemical properties of an active pharmaceutical ingredient in a drug product is critical for regulatory approval and therapeutic success. Physical constants such as specific gravity, refractive index, optical rotation obtained confirms that the Betel oil is pure and possesses the characteristics of a volatile oil

The dilution method and the disc diffusion method/agar well diffusion are used to determine the antimicrobial activity of the oil. The study showed that the Betel oil has a minimum inhibitory concentration for *Staphylococcus aureus* at 125 µg/mL, *Streptococcus pyogenes* 15.60 µg/mL, Candida *albicans* 250 µg/mL and Trichophyton *mentagrophytes* at1.95 µg/mL. The zone of growth inhibition of 67.50 mm for *S. aureus*, 90 mm for *S.pyogenes*, *C. albicans* and *T. mentagrophytes* demonstrated that Betel oil is a very effective antimicrobial agent.

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