P. amboinicus Under 22ºC and Soil Conditions in Lanao del Sur, Philippines: Its Phytochemical Properties

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ABSTRACT

The herb Cuban Oregano (*Plectranthus amboinicus*) belongs to the family *Lamiaceae* which can be found abundantly in the tropics and warm region of Africa, Asia, and Australia. This herb is widely used as a medicinal herb in the Philippines to treat conditions such as fever, cough, asthma, throat infections, nasal congestions, and for wound healing, which can be attributed to its natural phytochemical compounds. In this study, it aimed to determine the phytochemical components of Cuban Oregano (*Plectranthus amboinicus*) under the soil and climatic conditions of three different localities in Lanao del Sur such as: Marawi City, Lumbatan, and Ganassi. Treatments are T_1 (*Plectranthus amboinicus* Under Soil and Climatic Condition of Marawi City), T_2 (*Plectranthus amboinicus* Under Soil and Climatic Condition of Lumbatan), and T_3 (*Plectranthus amboinicus* Under Soil and Climatic Condition of Ganassi). Results of the study revealed that *Plectranthus amboinicus* contains *Flavonoids, Alkaloids, Phenolics, Phytosterols, and Tannins* as all the three treatments all resulted to positive (+) and it resulted to negative (-) for the R. Sugar. Based on the result, *Plectranthus amboinicus* from three different localities is safe from harmful phytochemical components and is safe for human consumption as for medicinal uses.

Keywords : *Plectranthus amboinicus*; Cuban Oregano; Alkaloids; Flavonoids; Phytosterols; Tannins; Phenolics

1 INTRODUCTION

HERBAL medicine has been used worldwide throughout the years for treatment and prevention of diseases. According to the World Health Organization, 80% of individuals around the world rely on herbal medicine for their primary health care due to its low cost and easy accessibility. Because of the increasing use of herbal medicine, concerns relating to its safety and efficacy are also increasing. Watchel-Galor and Benzie reported that regardless of the health benefits of herbs, it is still capable of producing a wide range of undesirable or adverse reactions some of which are capable of causing serious injuries, life-threatening conditions, and even death. This is due to lack of a systematic approach to assess their safety and effectiveness (Watchel-Galor and Benzie, 2011).

There are numerous herbs that can be found in the Philippines. According to Dela Cruz and Ramos, Philippines has 1,500 medicinal plants used by traditional healers and one of these is the Cuban oregano (*Plectranthus amboinicus*). *Plectranthus amboinicus*, also known as cuban oregano or suganda, is an erect, spreading, branched herb which can grow up to 50 cm tall and has fleshy stems up to 180 cm long. Its leaves are broadly ovate, fleshy, heart-shaped, and somewhat hairy with rounded, toothed margins (Maramba et al., 2018). *Plectranthus amboinicus* belongs to the family Lamiaceae (Arumugam et al., 2016).

Plectranthus amboinicus is widely used as medicine and as flavoring ingredient. Traditionally, *Plectranthus amboinicus* has been used in the Philippines as a cure for nasal congestion, cough, throat infections, headache, colic, dyspepsia, flatulence, rheumatism, myalgia, otalgia, and burns (Manlubatan et al., 2022; Maramba et al., 2018; Subhas et al., 2010).

This herb has therapeutical and nutritional properties attributed to its natural phytochemical compounds which are highly valued in the pharmaceutical industry (Arumugam et al., 2016). Studies have stated that the *Plectranthus amboinicus* contains a number of bioactive properties including anti-tumor, anti-oxidant, anti-microbial, anti-inflammatory, wound healing, antiepileptic, larvicidal, and analgesic activity. The essential oil extracted from the *Plectranthus amboinicus* has high amounts of bioactive compounds such as Carvacrol, Thymol, βCaryophyllene, α-Humulene, y-Terpinene, p-Cymene, α-Terpineol and β-Selinene which are beneficial to human body (Castillo and Gonzales, 1999; Singh et al., 2002; Murthy et al., 2009; Senthilkumar and Venkatesalu, 2010; Gonçalves et al., 2012; Bhatt and Negi, 2012).

IEEE-SEM, Volume 10, Issue 11, November-2022
ISSN 2320-9151

In this study, it aims to provide facts about the phytochemical properties and medical benefits of Cuban oregano (*Plectranthus amboinicus*) which may serve as a reference fo future studies.

2 Related Literature

2.1 Phytochemicals of Oregano (*Plectranthus amboinicus*)

Plectranthus amboinicus grows naturally and is used in various places around the world particularly in the warm regions of Africa, Asia, and Australia. This herb has therapeutic and nutritional properties attributed to its phytochemical compound (Arumugam et al., 2016) Several classes of phytochemicals are found in *P. amboinicus* including monoterepenoids, diterpenoids, triterpenoids, phenolics, flavonoid, etc. Yet, the chemical profile and the accumulation pattern of the phytochemicals of *P. amboinicus* in different parts of the plant and their essential oil content differs depending on different parameters such as the climate of the place where the plant grew, the geographical features, and the different stages of plant material collection (Swamy M.K., Sinniah U.R., 2015).

2.2 Volatile Constituents of *P. amboinicus*

It is said that there are 76 volatile constituents that can be found in tha essential oil obtained from the stem and leaves of *P. amboinicus*. Essential oils accumulated from the stem and leaves of *P. amboinicus* contained a copious amount of the two major phenolic compounds, particularly, Carvacrol and Thymol. Essential oil of *P. amboinicus* grown in India, is rich with carvacrol, thymol, eugenol, chavicol, ethyl salicylate (Dutta, S., 1959). Eucalyptol is also present in *P. amboinicus'* leaves when it was extracted through steam distillation and solid phase micro extraction methods. (Knab et al., 2009). In the western Ghats region of north west Karnataka India, a region of *P. amboinicus* detected 77% of Carvacrol, followed by 5.74% of betacaryophyllene, 3.72% of caryophyllene oxide, etc. (Joshi et al., 2011).

2.3 Thymol

Thymol is a volatile compound that can be found and extracted in several species mainly in Thymus vulgaris L., commonly known as thyme, and other plants such as Ocimum gratissimum L., Origanum L., Carum copticum L., different species of the genus Satureja L., Oliveria decumbens Vent, and many others (Escobar et al., 2020). Thymol or C10H14O in chemical formula, has various uses and it is widely used in the pharmaceutical and food preservative applications. Thymol based motifs are known to show broad of wide spectrum of biological activities including antibacterial, anticancer, antitumor, anti-inflammatory, antimicrobial, and others (Shankar et al., 2019). Thymol and thyme-contained oils are nontoxic to animals, aquatic lives, and humans, however, at high concentrations, thymol can irritate the skin and lungs.

In an experiment conducted by Baslas and Kumar (1981) it is found that the oil obtained in *P. amboinicus* by steam distillation contained 41.3% of Thymol along with other compounds such as a-pinene (3.20 %), b-pinene (2.50 %), bcaryophyllene (4.20 %), methyl eugenol (2.10 %) and such. Meanwhile in Haque (1988) experiment, Thymol was shown to be the principal component of the oil obtained from *P. amboinicus* with 79.6%. In Manjamalai and Berlin-Grace (2012, 2013) reported that the major volatile compounds that can be found in *P. amboinicus* are Thymol, Carvacrol, cis-Caryophyllene, trans-Caryophyllene, and p-cymene. In another report by Tewari et al. (2012) they found 16 identified constituents of *P. amboinicus* including thymol with 83-89% of the extracted oil from the plant.

2.4 Carvacrol

Carvacrol or $C_{10}H_{14}O$ in chemical formula is a phenolic monoterpenoid that is found in several species particularly and abundantly in the essential oils of oregano (*oreganum vulgare*) and thyme (*Thymus vulgaris*) (Sharifi-Rad et al., 2018). Currently, Carvacrol has various uses including as food preservatives and as food flavoring ingredients at low concentrations, fragrance ingredients at cosmetic formulations, and recently, in some considerable researches, it is found that carvacrol has a potential to be used in clinical applications (Suntres et al., 2013) Carvacrol contains a wide range of bioactivities commonly accepted for useful for clinical applications such antimicrobial, antioxidant, and anticancer activities (Sharifi-Rad et al., 2018). Carvacrol, an isomer of thymol, is found in essential oils isolated from oregano, thyme and Coleus Amboinicus. Like thymol, carvacrol also displays antimicrobial activity (Wadikar D. and Patki P., 2016).

In Joshi et al. (2011) studies, it reveals that 77% of essential oil of the aerial parts of Coleus Amboinicus (CA) or *P. amboinicus* herb was constituted by carvacrol followed by betacaryophyllene with 5.74%, etc. In Pino et al. (1989) investigation, they identified 20 components including terpene hydrocarbons and 7 oxygenated compounds and the oil contained about 64% of carvacrol. In another report by Mallavarapu et al. (1999) they analyzed the essential oils of CA distilled in different seasons and the oils were found to contain carvacrol as one of the major constituents.

2.5 Eugenol

Eugenol, a volatile bioactive naturally occurring phenolic monoterpenoid, belongs to phenylpropanoids class of natural products. It is usually found in variety of aromatic herbal plants such as clove, tulsi, cinnamon, nutmeg, and pepper, but mainly isolated from clove plant (Eugenia caryophyllata). Eugenol is well known for its diverse applications in various fields such as pharmaceutical, food, flavor, cosmetic, agricultural, and numerous other industries. Eugenol is well recognized for its pharmacological properties, viz. antimicrobial, anticancer, antioxidant, antiinflammatory, and analgesic. Different derivatives of eugenol are used in medication as a local anesthetic and antiseptic. Regardless of numerous applications, eugenol also shows various side effects particularly if taken in excess than the recommended dosage. It

IEEE-SEM, Volume 10, Issue 11, November-2022
ISSN 2320-9151

may cause nausea, dizziness, convulsions, and rapid heartbeat. Therefore, the aim of this chapter is to discuss the sources, methods of extraction and characterization, bioavailability, chemistry, mechanism of action, health benefits, pharmacological, safety and toxicology of eugenol (Sharma, Gohain, 2022).

Eugenol chemical structure is related to phenol. However, the toxicity does not include corrosive activities of phenol; ingestion results in vomiting, gastroenteritis, and secretion of mucin, and the resulting systemic toxicity is similar to phenol in some extent. There is no study demonstrating acute toxic effects of eugenol by occupational exposure. There are few studies in humans reported with accidental ingestion of eugenol; toxic effects were observed in liver, lung, and nervous system as discussed in mechanisms of toxicity (Sellamuthu, 2014).

In the study of Dutta (1959) in India, *Plectranthus amboinicus* essential oil was reported to possess volatiles such as Carvacrol (43.1%), Thymol (7.2%), Eugenol (6.4%), Chavicol (5.3%) and Et-salicylate (3.2%), which varied from the constituents observed by Baslas and Kumar (1981) with Thymol (41.30%), Carvacrol(13.25%), 1,8-Cineole (5.45%), Eugenol (4.40%) and βCaryophyllene (4.20%).

2.6 Beta-caryophyllene

β-Caryophyllene is generally the most common sesquiterpene in cannabis, and the most abundantly produced terpene in Nature. Over the last decade, it has gained scientific attention after discovering that it can directly activate cannabinoids receptors. Basically, the β-Caryophyllene terpene acts as a cannabinoid. The Terpene Beta-Caryophyllene is present in black pepper, oregano, basil, and many other herbs and spices. Beta-Caryophyllene molecular structure is unique; it is more significant than other terpenes and contains a rare cyclobutene ring not found in any other cannabis terpene. Beta-caryophyllene has potent anti-inflammatory, antimicrobial, antibacterial, and antioxidant properties. It is known to help relieve anxiety and pain, reduce cholesterol, prevent Osteoporosis, and treat seizures. Also, some research has shown that it may help against certain neurodegenerative diseases and cancers. Beta-caryophyllene can reduce inflammation in the brain and chemicals that cause oxidative stress associated with inflammation.

2.7 Non-Volatile Chemical Constituents of *P. amboinicus*

According to literature review of Arumugam G., Swamy M.K., Sinniah U.R. (2016), a total of 30 non-volatile constituents have been found from *P. amboinicus* and these includes phenolic acids, flavonoids, monoterpene hydrocarbons, oxygenated monoterpene, etc.

2.8 Flavonoids

Flavonoids are a class of polyphenol and are group of natural substances that are found in plants, fruits, vegetables, roots, grains, bark, stem, flower, herbs, tea and wine (Panche et al., 2016). Flavonoids can be found in almost all fruits, vegetables, and herbs; they at least contain a certain amount of flavonoid. Flavonoids have also become an extremely important in several fields such as nutraceutical, pharmaceutical, medicinal and cosmetic applications due to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic nature together with their capacity to modulate key cellular enzyme function or enzyme inhibition (Panche et al., 2016). Some flavonoids have antibacterial and antifungal properties, they also play a role in protecting the plants from microbe and insect attacks; more importantly, the consumptionof foods containing flavonoids has been linked to numerous health benefits (Kesarkar et al., 2009).

Kaliappan and Viswanathan (2008) reported in their phytochemical study that various flavonoids like quercetin, apigenin, luteolin, salvigenin, and genkwanin have been found in the leaves of *P. amboinicus*. In Bhatt et al. (2013) analyzation of the phytochemicals of *P. amboinicus*, particularly in methanolic stem extracts of *P. amboinicus*, their study revealed the existence of a total flavonoids (26.6 mg rutin equivalent (RE)/g extract) and other components. In Asiimwe et al. (2014) evaluation of the aqueous leaf extracts of *Plectranthus amboinicus* is reported to contain flavonoids.

2.9 Phenolic Acids

Phenolic acids also known as phenol carboxylic acids are class of aromatic acids. They are aromatic secondary plant metabolites widely spread throughout the plant kingdom; recent interest in phenolic acids stems from the potential protection they offer against oxidative damage diseases such as: coronary heart disease, stroke, and cancers, when consumed in fruits and vegetables (Robbins, 2003). Furthermore, phenolic acids are found ubiquitously and well documented for other health protective effects like antimicrobial, anticancer, anti-inflammatory, antimutagenic etc. The contribution emphasizes on the phenolic acids' potential in drug discovery (Kumar and Goel, 2019). Bhatt et al. (2013) study revealed the existence of total phenolic content (49.9 mg gallic acid equivalent (GAE)/g extract).

2.10 Monoterpenes

Monoterpenes are a class of terpenes that consist of two isoprene units and have the molecular formula C10H16. Monoterpenes may be linear (acyclic) or contain rings (monocyclic and bicyclic). Modified terpenes, such as those containing oxygen functionality or missing a methyl group, are called monoterpenoids. Monoterpenes and monoterpenoids are diverse. They have relevance to the pharmaceutical, cosmetic, agricultural, and food industries (Eberhard, 2006). Many monoterpenes are volatile compounds and some of them are well-known fragrants found in the essential oils of many plants (Loza-Tavera ,1999). For example, camphor, citral, citronellol, geraniol, grapefruit mercaptan, eucalyptol, ocimene, myrcene, limonene, linalool, menthol, camphene and pinenes are used in perfumes and cosmetic products. Limonene and perillyl alcohol are used in cleaning products (Laszlo, 2007). According to a review, several studies showed "that some monoterpenes (e.g., pulegone, menthofuran, camphor, and limonene) and sesquiterpenes (e.g., zederone, germacrone) exhibited liver toxicity" and

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ISSN 2320-9151

that i.a. intensive research on terpenes toxicity is needed (Caputi et al., 2011) Monoterpenes are found in the essential oils of many plants including fruits, vegetables, and herbs. They prevent the carcinogenesis process at both the initiation and promotion/progression stages. In addition, monoterpenes are effective in treating early and advanced cancers. Monoterpenes such as limonene and perillyl alcohol have been shown to prevent mammary, liver, lung, and'other cancers. These compounds have also been used to treat a variety of rodent cancers, including breast and pancreatic carcinomas. In addition, in vitro data suggest that they may be effective in treating neuroblastomas and leukemias (Crowell P.L.,1994).

2.11 Tannins

The tannin compounds that are present in some plants, provides them protection against predation. This also makes them to act as pesticides and to regulate the growth of plants. The term tannin is widely applied to a complex large biomolecule of polyphenol nature having sufficient hydroxyls and other suitable groups such as carboxyl to form strong complexes with various macromolecules (Navarrete, 2013). In this present review, tannins were detected in most plant species like peel and juice of Citrus medica,mango (Mangifera indica L.) leaves, Avocado fruit (PerseaAmericana), Dioscorea alata leaf, of Leucas aspera L. leaf and root, Ocimum gratissimum Linn leaf, Rhamnus prinoides root, extract of Rhizomes, Zingiber officinale and Curcuma longa and also for different solvent give different response for the same plant species like Bersama abyssinica leaf, Flax seeds, Nigella sativa, Ruta chalepensis leaves, and Syzygium guineense and not totally detected in part of plants like Lepidium sativum seeds and love Gilbetii root. Tannins are generally used in the tanning process and used as healing agents in inflammation, burn, piles, and gonorrhea (Boroushaki et al., 2016).

3 RESEARCH METHODOLOGY

3.1 Research Design and Locale of the Study

Experimental research was used in this study, which modifies and controls variables based on the scientific method. The experiment was conducted in a laboratory setting. Researchers assessed the phytochemical properties of *Plecthrantus amboinicus* under soil and climatic conditions of the three aforesaid areas in Lanao del Sur.

3.2 Phytochemical Properties. The Phytochemical analysis of *Carica* papaya was performed for the presence of alkaloids, carbohydrates, amino acids, glycosides, protein, phenolic compounds, and tannins from respective solvents such as hexane, ethyl acetate, methanol, and ethanol, according to standard procedure.

3.2.1 Phytochemical Screening. The concentrated concentrate of chosen plant will be exposed to various compound test for the location of various phytoconstituents utilizing standard techniques.

3.2.2 Test for alkaloids. 1 ml of the sample solution, few drops were added along the sides of the test tube. The appearance of a reddish-brown precipitate indicates the presence of alkaloids.

3.2.3 Test for Carbohydrates. 1 ml of the sample solution, few drops were added and heated for 2 min. The appearance of a colored precipitate indicates the presence of carbohydrates.

3.2.4 Test for Amino acids. 1 ml of the sample solution, two drops were added. The formation of purple color indicates the presence of amino acids.

3.2.5 Test for Glycosides. 1 ml of sample solution 1 ml of glacial acetic acid, few drops of ferric chloride and concentrated sulfuric acid were added. The appearance of a reddish-brown ring at the junction of liquids indicates the presence of glycosides.

3.2.6 Test for Phenolic compounds and tannins. 1 ml of extract, few drops of neutral 5% ferric chloride were added. The appearance of a dark green color indicates the presence of phenolic and tannin compounds.

3.2.7 Test for Protein. 1 ml of extract, one drop of 2% copper sulfate and 1 ml of ethanol and potassium hydroxide were added. The presence of the pink color of the ethanolic layer indicates the presence of protein.

3.2.8 Test for Saponins. 1 ml of extract, few drops of distilled water were added and shaken vigorously. The appearance of foam indicates the presence of saponins.

3.2.9 Test for Quinones. 1 ml of extract, few drops of concentrated hydrochloric acid were added. The formation of a yellow precipitate indicates the presence of quinones.

IEEE-SEM, Volume 10, Issue 11, November-2022
ISSN 2320-9151

3.2.10 Test for Oxalate. For 1 ml of extract, few drops of glacial acetic acid were added. The appearance of greenish-black coloration indicates the presence of oxalate.

3.2.11 Test for Anthocyanins. 1 ml extract, 2 ml of hydrochloric acid and 1 ml of ammonia were added. The color change from pink-red to blue-violet indicates the presence of anthocyanins.

4 Result and Discussion

Table 1: Summary of Results on Phytochemical Analysis of *Plectranthus amboinicus*

Treatments		Phytochemical Components					
		Alkaloids	Flavonoids	Phytosterols	Tannins	Phenolics	R. Sugar
T_1	*Plectranthus amboinicus* Under Soil and Climatic Condition of Marawi City	+	+	+	+	+	-
T_2	*Plectranthus amboinicus* Under Soil and Climatic Condition of Lumbatan	+	+	+	+	+	-
T_3	*Plectranthus amboinicus* Under Soil and Climatic Condition of Ganassi	+	+	+	+	+	-

4.1 Phytochemical analysis of Cuban Oregano (*Plectranthus amboinicus*)

Based on the result of the study, Table 1 shows a positive result of Alkaloids, Flavonoids, Phytosterols, Tannins and Phenolics in the T_1 (*Plectranthus amboinicus* Under Soil and Climatic Condition of Marawi City), in T_2 (*Plectranthus amboinicus* Under Soil and Climatic Condition of Lumbatan), and in T_3 (*Plectranthus amboinicus* Under Soil and Climatic Condition of Ganassi. On the other hand, it resulted negative for the Reducing Sugar (R. Sugar) in all of the three treatments tested.

Alkaloids has a diverse use in the field of medicine and pharmacology and is used as toxins, stimulants, pharmaceuticals or recreational drugs, including caffeine, atropine and cocaine (Ranjitha et al., 2015). Meanwhile, Phytosterols are compound that can be found in plant that has a various health benefits including reducing one's cholesterol level when taken as part of one's healthy diet, reduce the risk of cardiovascular disease, heart attack and stroke (Cleveland Clinic, 2022). In terms of Flavonoids, they have played an important role in various fields such as in pharmaceutical and medicinal field due to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic nature (Panche et al., 2016), more importantly, the consumption of foods containing flavonoids has been linked to numerous health benefits (Kesarkar et al., 2009). Phenolic's phenolic acids offers a potential protection against oxidative damage diseases such as: coronary heart disease, stroke, and cancers, when consumed in fruits and vegetables (Robbins, 2003). Lastly, Tannins are commonly used as healing agents in inflammation, burn, piles, and gonorrhea (Boroushaki et al., 2016). Thus, it shows that *P. amboinicus* is safe and will help cure some diseases.

5 Conclusion

Thus, based on the study conducted, it can be stated that the herb *Plectranthus amboinicus* is safe from harmful phytochemical components and is safe for human and folk medicinal uses.

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