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PRELIMINARY PHYTOCHEMICAL SCREENING OF *Pimenta racemosa var. racemosa* (MYRTACEAE) FROM TÁCHIRA – VENEZUELA

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Abstract

Pimenta racemosa var. *racemosa* (Mill.) J. W. Moore, commonly known as Bay-rum, Malagueta, Pepita species and pepper, is a Caribbean tree, belonging to Myrtaceae family, with a special interest in their leaves for the production of essential oil well known as "Bay-Rum". According to literature consulted there are no reports either for phytochemical or pharmacological activities with respect to extracts of this species. The aim of this study was to evaluate extracts from aerial parts (leaves, fruits, stems and bark) and roots of *P. racemosa* var. *racemosa* collected in April 2012, in order to perform a preliminary phytochemical screening. All extracts were obtained by maceration, and phytochemical screening was carried out by simple colorimetric and TLC techniques. Results indicated mainly the presence of terpenoids, steroids, saponins, phenolic nuclei, glycosides, tannins, and quinines. This information no only supports the use of this plant in traditional medicine but might be useful for further chemical and biological investigations.

Keywords: phytochemical screening, *Pimenta racemosa* var. *racemosa*, terpenoids, steroids, phenolic nuclei.

Introduction

Genus *Pimenta* belongs to family Myrtaceae, comprises 21 species including several varieties, is typical from tropical America (1). In Venezuela is only represented by *P. racemosa* (Mill.) JW Moore (*P. acris* Kostel), and is distributed in Federal District, Falcón, Lara, Merida, Nueva Esparta, Sucre, Tachira and Zulia states (2), commonly known as: Bay-rum, Malagueta, Pepita and pepper species, is cultivated as an ornamental (3), and used in folk medicine for the treatment of various diseases, such as fever, rheumatism, toothache, abdominal pain, pneumonia, colds (4-5), pectoral angina, diarrhea, incontinence, stroke, anti-inflammatory and analgesic properties. Furthermore, its dry wood is used in fence posts because of their resistance against termites (5).

Among pharmacological effects reported for different *Pimenta* species include: insect repellent (6), antidermatophytic (7), antinociceptive (4, 8-9), anti-inflammatory (4, 10), antipyretic (9), antimicrobial (11-19), antioxidant (20-26), anti-cancer (23), antimutagenic (22), anti-hemorrhagic bleeding and cobra venom (27), central nervous system depressant (8, 28-29), hypoglycemic (30), hypotensive (8, 28-29), inhibitor of histone acetyl transferase enzyme (31), and inhibitor of enzyme histidine decarboxylase (32).

Pimenta genus chemistry has also been explored for leaves and fruits and phytochemical studies have primarily led to the isolation of various tannins, phenolic compounds, flavonoids, one triterpene saponin and a structural variety of volatile components such as monoterpenes, sesquiterpenes and phenylpropenes (33).

P. racemosa var. *racemosa*, is an aromatic tree of tropical origin. To date, studies of this species have been focused on the leaves due to the content of volatile essences, once distilled, are used in the manufacture of cosmetics, especially in formulations such as after shave lotions, soaps, colonies and hair treatments (34-35), however to date, literature has not reported studies on phytochemicals or pharmacological activities with respect to extracts of this species, thus, it is expected that extracts might be a source of similar secondary metabolites reported for gender. The aim of this study is to confirm the presence or absence of these metabolites by performing a detailed phytochemical screening that might be useful in further phytochemical investigations.

Materials and methods

Plant material

Leaves, fruits, stems (fine and coarse), bark and roots of *P. racemosa* var. *racemosa* were collected in April 2012, near Sector "Los Corredores de la Palmita" Junín Municipality, Rubio town, located at southwestern Táchira state, Venezuela, altitude 859 m.s.n.m. Botanical identification was carried out by Dr. Leslie R. Landrum, Herbarium Curator, School of Life Sciences, Arizona State University, USA. Specimens collected in field are sheltered in MERF Herbarium of the Faculty of Pharmacy and Biomedical Sciences, University of Los Andes (BC-01 code), Venezuela and in ASU Arizona Herbarium of Arizona State University (ASU0075448 code), USA.

Extraction

Plant material (leaves, fruit, thin stems, thick stems, bark and roots) was dried in an oven with air circulation at 40 °C for 5 days , then was pulverized to yield 4615g leaves (L), 566g fruits (F), 8160g thin stems (TS), 16105g thick stems (TSt), 5572g cortex (C) and 3905g roots (R). All extracts were extracted by soaking with isopropyl alcohol (1:4 m/v) in 4 periods of 21 days each, thereby achieving an exhaustive extraction. Extracts obtained were filtered using filter paper (Double Rings No. 203) and concentrated under reduced pressure at 30°C to remove the remaining isopropyl alcohol. After concentration every extract was weighted yielding 604.11 g (L), 58.17 g (F), 434,82 g (TS), 508,15 g (TSt), 265,44 g (C) y 109,52 g (R).

Phytochemical screening

Crude extracts of L, F, TS, TSt, C and R of *P. racemosa* var. *racemosa* were phytochemically evaluated to determine the presence of chemical constituents using standard procedures, which are described below:

Testing for Alkaloids: each extract (10 mg) was dissolved in 2 mL of hydrochloric acid 5%, after mixing and filtered, three aliquots were taken. Drops of Wagner, Mayer and Dragendorff reagents were added to each. A red-brown precipitate (Wagner), yellowish white precipitate (Mayer) and red-orange precipitate (Dragendorff) indicated the presence of such metabolites (36).

Testing for Coumarins: 10 mg of each sample were

added to 0.5 mL of ethanol along with 2 drops of concentrated ammonium hydroxide. If examination under UV light at a wavelength of 365nm shows the presence of blue or green fluorescence might be indicative of a positive result (36).

Testing for Glycosides: 10 mg of each extract were dissolved in 1 mL of distilled water followed by 5 drops of aqueous sodium hydroxide. A yellow colour indicated the presence of glycosides in the extract (41).

Testing for Cardiotonic glycosides:

- Keller-Killiani reaction: 10 mg of each extract were dissolved in Keller's reagent and then 5 drops of concentrated sulfuric acid were added. The occurrence of brown ring between the two phases formed is indicative of deoxy glycosides nuclei presence. (36)
- Legal Reaction: 3 drops of pyridine, 1 drop of sodium nitroprusside solution (aqueous) 5% and 3 drops of 2N sodium hydroxide were added to 10 mg of each extract. Intense red coloration indicated the presence of cardenolides, or α,β–unsaturated lactones nuclei (36).

Testing for Flavonoids: Shinoda, Pew's, 10 % sodium hydroxide and 2-aminoethyl diphenyl borate assays were performed in order to determine the presence of these metabolites.

- Shinoda test: 1 mL of absolute ethanol and 3 drops of concentrated hydrochloric acid were added to 10 drops of diluted extract in isopropyl alcohol. Formation of red color indicated the presence of aurones and chalconnes. In cases where no colour change was observed, pieces of metallic magnesium were placed. The formation of orange, red or magenta coloration indicated the presence of flavones, flavonols and flavones, respectively (36).
- Pew's test: 2 mg of zinc powder and 5 drops of 5N hydrochloric acid were added to 1 mL of each diluted extract in isopropyl alcohol. The presence of red, pink or coffee color indicated the existence of dihyflavones, flavonones, and dihydrochalcones, respectively (36).
- Test with 10% sodium hydroxide: 3 drops of sodium hydroxide 10% were added to 1 mL of diluted extract in isopropyl alcohol. Formation of yellow-red, coffee-orange, purple-red or blue coloration indicated the presence of xanthones and/or flavones, flavonols, chalcones and anthocyanins, respectively (36).
- Test with 2-aminoethyl diphenylborate: Methanolic solutions of each extract were analyzed by thin layer chromatography (TLC) on silica gel 60 F254 plates, at room temperature. Solvent system was chloroform–methanol formic acid–water (3:6:1:1). Visualization was performed under UV-Vis light (350nm) before and after staining with 1% methanolic 2-aminoethyl diphenylborate reagent. Appearance of yellow fluorescence, after spraying with reagent was indicative of flavonoids nuclei (38-39).

Testing for steroids and/or triterpenoids: Salkowski, Rosenthaler and Lieberman-Bouchard assays were carried out (36).

- Salkowski test: 2 mL of chloroform and 1 mL of concentrated sulfuric acid were added to 10 drops of the extract dissolved in isopropyl alcohol, slowly until double phase formation. The presence of a reddish-brown color in the middle layer was indicative of steroidal ring.
- Rosenthaler test: 3 drops of Rosenthaler reagent and 2 drops of concentrated sulfuric acid were added to 2 mL of the extract dissolved in isopropyl alcohol. Formation of violet color in the middle layer was indicative of terpenoids.
- Lieberman-Bouchard test: 1 mL of anhydrous acetic acid and 3 drops of concentrated sulfuric acid were added to 2 mL of the extract dissolved in isopropyl alcohol. After 5 minutes a blue-green color middle layer was indicative of sterols, but a pink, red, magenta or violet color revealed the presence of terpenoids.

Testing for Quinones and Anthraquinones: Presence of such metabolites was determined by Borntraguer, ammonium hydroxide, concentrated sulfuric acid (36) and benzene assays (40).

-Borntraguer test: 10 mg of each extract were dissolved in 3 mL of distilled water and filtrated. After filtration 3 mL of 5% potassium hydroxide solution were added to each. The mixture was heated to boiling for 3 minutes. Alkaline solution was allowed to cool down and then extracted with 3 mL of chloroform. Organic phase was separated and shaken with 2 mL of 5% potassium hydroxide solution. Occurrence of red color in alkaline phase indicated the presence of quinones. Those samples showing yellow colour with green fluorescence where treated with one drop of 6% hydrogen peroxide, formation of red colour was considered positive for anthrones derivatives.

- Test with ammonium hydroxide: One drop of concentrated ammonium hydroxide was added to 10 mg of each extract, previously dissolved in isopropyl alcohol. After two minutes, formation of red colour indicated the presence of anthraquinone.
- Test with sulfuric acid: One drop of concentrated sulfuric acid was added to 10 mg of each extract dissolved in isopropyl alcohol. Formation of red color indicated the presence of quinones.
- Test with benzene: 1 mL of benzene was added to 10 mg of each extract dissolved in isopropyl alcohol, followed by stirring and filtration. 0.5 mL of 10% ammonia solution was added to the filtrate. This mixture was shaken; formation of pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of anthraquinones.

Testing for Tannins: 100 mg of each extract were dissolved in 10 mL of ethanol, and extracted with 25 mL of distilled water in boiling during 15 minutes. Once allowed to fresh at room temperature, 0.2 mL of 10% sodium chloride solution were added to mixture and filtered. The filtrate was divided into four equal portions in test tubes. 5 drops of 1% gelatin solution were added to first portion, 5 drops of gelatin - salt solution (1% gelatin + 10% salt) were added to second portion, 4 drops of 10% ferric chloride solution were added to third portion, and 3 drops of 1% potassium ferricyanide solution were added to fourth portion. Precipitation observed after addition of either second or third reagent was indicative of the presence of tannins. Those samples showing grayish-black or black-blue colour after addition of third reagent indicated the presence of tannins with catechol or pyrogallol nuclei, respectively. Samples turning to blue colour after fourth reagent revealed the presence of phenolic compounds (41).

Testing for Phenols: 10 mg of each extract were dissolved in 1 mL of ethanol, then 2 mL of distilled water was added followed by 4 drops of ferric chloride aqueous solution 10% w/v. Formation of a blue or green color indicated the presence of phenols (42).

Testing for Saponins:
- Test Foam height (without sodium bicarbonate): 1 mL of distilled water were added to 10 drops of the extract dissolved in isopropyl alcohol (20 mg/mL) in a test-tube, shaken vigorously to froth, then allowed to stand for 30 minutes. Saponin content was measured as follows: no froth (absence); froth less than 3 mm high (poor); froth 6 mm high (moderate) and froth greater than 8 mm high (abundant) (36).

- Foam Test (with sodium bicarbonate): 1 mL of distilled water and 1 drop of sodium bicarbonate saturated solution were added to 5 drops of the extract dissolved in isopropyl alcohol (20 mg/mL) in a test-tube and shaken vigorously during 3 minutes. Formation of honeycomb shaped foam indicated the presence of saponins (42).

Results and Discussion

Preliminary phytochemical screening from organic extracts (L, F, TS, TSt, C and R) of *P. racemosa* var. *racemosa* was carried out using various chemical assays in order to identify either the presence or absence of secondary metabolites such as alkaloids, coumarins, phenolic compounds, flavonoids, glycosides, quinones, saponins, tannins, steroids and triterpenoids

The results (Table 1) revealed abundant presence of phenolic nuclei and tannins in all extracts assayed while no alkaloid incidence was observed. In reference to glycosides cardiotonic, steroid, terpenoids, quinones, and flavonoids, these components are present in all extracts evaluated; the only difference might possible be the amounts between these. For instance, deoxy glycosides nuclei were abundant in L extract, moderate in F and C extracts while R extract showed low content. Cardenolides or α,β–unsaturated type lactones were in high concentration in TS, TSt and R extracts, moderate incidence in L and C extracts but minor presence in F extract. Steroids were well evident in L, TS, TSt, C and R extracts while terpenoids were abundant in L and TS respectively. Regarding flavonoids, the presence of flavones or xanthones type components were observed moderately in F extract; dihydrochalcones and/or flavonols in R, L, TSt and C. Quinones showed to be moderated in F, TS, TSt, C and R extracts. Moreover, saponins were present in five of the extracts assayed (L, TS, TSt, C, R), being R extract the most abundant between these followed by TSt.
Anthraquinone nuclei was observed abundant in TS, TSt extracts but moderated in R and C, respectively

According to previous investigations *P. dioica* and *P. pseudocaryophyllus* are composed mainly by flavonoids (27, 32, 44), tannins (23, 27, 44) and terpenes present as essential oils (7, 16,17, 43). Moreover, their fruits revealed the presence of phenylpropanoids (20), tannins (21) and phenolic compounds (51). *P. racemosa* var. *ozua* leaves revealed mainly the presence of lupeol (10) but leaves of *P. adenoclada* (46), *P. haitiensis* (47), *P. jamaicensis* (48), *P. obscura* (49), *P. racemosa* var. *grisea* (14, 45), *P. racemosa* var. *hispaniolensis* (45), *P. racemosa* var. *racemosa* (45, 50) and *P. racemosa* var. *terebinthina* (14) showed only terpene type components.

Comparing results from present investigation to previous reported for *P. dioica* and *P. pseudocaryophyllus*, occurrence of flavonoids, phenolic compounds, tannins and saponins were corroborated, however, other secondary metabolites such as glycosides, quinones, steroids and terpenoids were also observed in all extracts assayed.

Ever since no published works have been found regarding phytochemical studies on different parts of *Pimenta racemosa* var. *racemosa*, present investigation is consider as a qualitative type examination that reveals information about several possible nucleus present on this species that might be useful as reference for further investigations.

On the other hand, secondary metabolites observed in all extracts assayed in present investigation might be associated to possible biological activities. According to references consulted flavonoids have been associated to antimicrobial, anticancer, anti-inflammatory and antioxidant activities (42). Tannins have revealed antiviral, anti-inflammatory and antitumor properties (42). Saponins are used mainly as mild detergent, however has exhibited antioxidant, antifungal, anticancer among other activities (42). Triterpenes and steroids act as antiseptic, antibacterial and antifungals (52), quinones are used as anthelmintic, have laxative effects and are used in the treatment of fever, gonorrhea, leprosy and menstrual cramps (37). Polyphenols exhibit a wide range of biological activities, including anti-cancer, anti-inflammatory, antihypertensive, estrogenic, antioxidant and to prevent cardiovascular diseases (53).

Conclusions

Concerning *Pimenta* genus, flavonoids, tannins, phenolic compounds, saponins, and glycosides are the most common metabolites reported.

Specie assayed in present investigation showed to be consistent to previous reports. However, coumarins, glycosides and α,β-unsaturated ketones were also observed in several parts of the plant. According to references consulted for *P. racemosa* var. *racemosa* only essential oil composition has been published to date.

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References

1. Wilson PG. *Myrtaceae.* In: Kubitzki K, eds. The Families and Genera of Vascular Plants. Flowering Plants. Eudicots. Sapindales, Cucurbitales, Myrtaceae. Volume X. Springer Berlin Heidelberg. 2011: 212-271.
2. Holst BK. *Myrtaceae.* In: Hokche O, Berry PE, Huber O, eds. Nuevo Catalogo de la Flora Vascular de Venezuela. Caracas, DF: Fundación Instituto Botánico de Venezuela Dr. Tobías Lasser. 2008: 524.
3. Aristiguieta L. *Familias y Géneros de los Arboles de Venezuela*. Edición especial del Instituto Botánico, dirección de recursos naturales renovables. Ministerio de Agricultura y Cría. Caracas. Venezuela. 1973: 549.
4. García MD, Fernández MA, Álvarez A, et al. *Antinociceptive and anti-inflammatory effect of the aqueous extract from leaves of Pimenta racemosa var. ozua (Mirtaceae).* Journal of Ethnopharmacology, 2004; *91*(1): 69-73.
5. Kirk, TK. *Tropical Trees of Florida and the Virgin Islands: A guide to identification, characteristics and uses*. First Edition. Sarasota, FL. Pineapple Press Inc. 2009: 108.
6. González-Quevedo M, Báez F, Céspedes A, et al. *Uso de la crema repelente de Pimenta dioica por combatientes de una unidad militar.* Revista Cubana de Medicina Militar, 1997; *26*(2): 94-97.
7. Hernández L, Rodríguez J, García MD, et al. *Actividad antidermatofítica in vitro de aceites esenciales.* Revista Cubana de Plantas Medicinales, 2003; 8(2): 0-0.
8. Suárez A, Ulate G, Ciccio JF. *Efectos de la administración aguda y subaguda de extractos de Pimenta dioica (Myrtaceae) en rata albinas normotensas e hipertensas.*

Rev. biol. trop, 1997; 44(3/1): 39-45.
10. Benítez A, Tillán J, Cabrera Y. Actividad analgésica y antipirética de un extracto fluido de Pimenta dioica (L.) y evaluación de su toxicidad aguda oral. Revista Cubana de Farmacia, 1998; 32(3): 198-203.
11. Fernández A, Álvarez A, García MD, et al. *Anti-inflammatory effect of Pimenta racemosa var. ozua and isolation of the triterpene lupeol.* Il Fármaco, 2001; 56(4): 335-338.
12. Bara MTF, Vanetti MCD. *Estudo da atividade antibacteriana de plantas medicinais, aromáticas e corantes naturais.* Revista Brasileira de Farmacognosia, 1998; *7*(1): 22-34.
13. Hammer KA, Carson CF, Riley TV. *Antimicrobial activity of essential oils and other plant extracts.* Journal of applied microbiology, 1999; *86*(6): 985-990.
14. Burt SA, Reinders RD. *Antibacterial activity of selected plant essential oils against Escherichia coli O157: H7.* Letters in applied microbiology, 2003; 36(3): 162-167.
15. Sáenz MT, Tornos MP, Álvarez A, et al. *Antibacterial activity of essential oils of Pimenta racemosa var. terebinthina and Pimenta racemosa var. grisea*. Fitoterapia, 2004; *75*(6): 599-602.
16. Romero CD, Chopin SF, Buck G, et al. *Antibacterial properties of common herbal remedies of the southwest.* Journal of ethnopharmacology, 2005; *99*(2): 253-257.
17. Lima MEL, Cordeiro I, Young MCM, et al. *Antimicrobial activity of the essential oil from two specimens of Pimenta pseudocaryophyllus (Gomes) LR Landrum (Myrtaceae) native from São Paulo State-Brazil,* Pharmacologyonline, 2006; *3*: 589-593.
18. Oussalah M, Caillet S, Saucier L, et al. *Antimicrobial effects of selected plant essential oils on the growth of a Pseudomonas putida strain isolated from meat.* Meat science, 2006; *73*(2): 236-244.
19. Oussalah M, Caillet S, Saucier L, et al. *Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157:H7, Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. Food Control, 2007; *18*(5): 414-420.
20. Paula JAM, Paula JR, Pimenta FC, et al. *Antimicrobial activity of the crude ethanol extract from Pimenta pseudocaryophyllus.* Pharmaceutical Biology, 2009; 47(10): 987-993.
21. Kikuzaki H, Hara S, Kawai Y, et al. *Antioxidative phenylpropanoids from berries of Pimenta dioica*. Phytochemistry, 1999; *52*(7): 1307-1312.
22. Kikuzaki H, Sato A, Mayahara Y, et al. *Galloylglucosides from berries of Pimenta dioica.* Journal of natural products, 2000; 63(6): 749-752.
23. Ramos A, Visozo A, Piloto J, et al. *Screening of antimutagenicity via antioxidant activity in Cuban medicinal plants.* Journal of Ethnopharmacology, 2003; 87(2): 241-246.
24. Marzouk MS, Moharram FA, Mohamed MA, et al. *Anticancer and antioxidant tannins from Pimenta dioica leaves*. Zeitschrift fur Naturforschung C, 2007; 62(7/8): 526.
25. Kluth D, Banning A, Paur I, et al. *Modulation of pregnane X receptor-and electrophile responsive element-mediated gene expression by dietary polyphenolic compounds.* Free Radical Biology and Medicine, 2007; 42(3): 315-325.
26. Jirovetz L, Buchbauer G, Stoilova I, et al. *Spice plants: chemical composition and antioxidant properties of Pimenta Lindl. essential oils. Part 2: Pimenta racemosa (Mill.) J.W. Moore leaf oil from Jamaica.* Ernaehrung/Nutrition, 2007; 31/NR. 7/8: 293-300.
27. Dearlove RP, Greenspan P, Hartle DK, et al. Inhibition of protein glycation by extracts of culinary herbs and spice. Journal of medicinal food, 2008; 11(2): 275-281.
28. Castro O, Gutiérrez JM, Barrios M, et al. *Neutralización del efecto hemorrágico inducido por veneno de Bothrops asper (Serpentes: Viperidae) por extractos de plantas tropicales.* Revista de Biología Tropical, 1999; 47(3): 605-616.
29. Suárez A, Ulate G, Ciccio JF. *Cardiovascular effects of ethanolic and aqueous extracts of Pimenta dioica in Sprague-Dawley rats.* Journal of Ethnopharmacology, 1997; 55(2): 107-111.
30. Suárez A, Ulate G, Ciccio JF. *Hypotensive action of an aqueous extract of Pimenta dioica (Myrtaceae) in rats.* Revista de Biología Tropical, 2000; 48(1): 53-58.
31. Barbosa-Filho JM, Vasconcelos TH, Alencar AA, et al. *Plants and their active constituents from South, Central, and North America with hypoglycemic activity.* Revista Brasileira de Farmacognosia, 2005; 15(4): 392-413.
32. Lee YH, Hong SW, Jun W, et al. *Anti-histone acetyltransferase activity from allspice extracts inhibits androgen receptor-dependent prostate cancer cell growth.* Bioscience, biotechnology, and biochemistry, 2007; 71(11): 2712-2719.
33. Nitta Y, Kikuzaki H, Ueno H. *Inhibitory activity of Pimenta dioica extracts and constituents on recombinant human histidina descarboxilase*. Food Chemistry, 2009; 113: 445-449.
34. Paula JAM, Reis JB, Ferreira LHM, et al. Gênero *Pimenta: aspectos botânicos, composição química e potencial farmacológico; Pimenta genus: botanical aspects, chemical composition and pharmacological potential*. Revista Brasileira de Plantas medicinales, 2010; 12(3): 363-379.
35. Weiss EA. *Spice Crops.* CABI Publishing. USA. 2002: 131-132.
36. Boning C R. *Florida's Best Herbs and Spices: Native and Exotic Plants Grown for Scent.* First edition. USA. Pineapple Press Inc. 2010: 32-33.
37. Domínguez X. *Métodos de Investigación Fitoquímica*. México. Limusa, S.A., 1979: 41-43.
38. Marcano D, Hasegawa M. *Fitoquímica orgánica.* Consejo de desarrollo científico y humanístico. Universidad Central de Venezuela. Caracas, Venezuela. 2002: 57-59.
39. Garrido G, Ortiz M, Pozo P. *Fenoles y flavonoides totales y actividad antioxidante de extractos de hojas de Lampaya medicinalis F. Phil.* Journal of Pharmacy & Pharmacognosy Research, 2013; 1(1): 30-38.
40. Tie-xin T, Hong W. *An image analysis system for thin-layer chromatography quantification and its validation.* Journal of chromatographic science, 2008; 46(6): 560-564.
41. Rajesh P, Latha S, Selvamani P, et al. *Phytochemical screening and toxicity studies on the leaves of capparis sepiaria Linn. (Capparidaceae).* J Clin Basic Pharma. 2010; 1(1): 41-46.
42. Orantes, S. *Tamizaje fitoquímico de la especie vegetal guatemalteca Quararibea yunckeri Standley Subsp. izabalensis W.S. Alverson ex Véliz (Bombacaceae).* Tesis de Licenciatura en Química. Universidad de San Carlos. Guatemala. 2010: 13.

43. *Shyamala-Gowri S, Vasantha K. Phytochemical screening and antibacterial activity of Syzygium cumini (L.) (Myrtaceae) leaves extracts. Int.J. PharmTech Res. 2010; 2(2): 1569-1573.*
44. *Tucker A, Maciarello M, Landrum L. Volatile leaf oils of Caribbean Myrtaceae. II. Pimenta dioica (L.) Merr. of Jamaica.* J essent oil res. 1991; 3(3): 195-196.
45. Paula JAM, Paula JR, Bara MTF, et al. *Estudo farmacognóstico das folhas de Pimenta pseudocaryophyllus (Gomes) LR Landrum-Myrtaceae.* Rev Bras Farmacogn, 2008; 18: 265-278.
46. Tucker A, Maciarello M, Adams R, et al. *Volatile leaf oils of Caribbean Myrtaceae. I. Three varieties of Pimenta racemosa (Miller) J. Moore of the Dominican Republic and the commercial bay oil.* J essent oil res. 1991; 3: 323-329.
47. Pino J, Bello A, Urquiola A. *The leaf oil of Pimenta adenoclata (Urb) Burret from Cuba.* J essent oil res. 2002; 14: 400-401.
48. Tucker A, Maciarello M, Adams R, et al. *Volatile leaf oils of Caribbean Myrtaceae. III. Pimenta haitiensis* (Urban) Landrum of the Dominican Republic. J essent oil res. 1991; 3(6): 471-473.
49. Tucker A, Maciarello M, Landrum L. *Volatile leaf oils of Caribbean Myrtaceae. IV. Pimenta jamaicensis (Britton & Harris) Proctor of Jamaica.* J essent oil res. 1992; 4(1): 193-194.
50. Tucker A, Maciarello M, Landrum L. *Volatile leaf oils of Caribbean Myrtaceae. V. Pimenta obscura Proctor of Jamaica*. J essent oil res. 1992; 4(2): 195-196.
51. Contreras-Moreno B, Rojas J, Celis M, Rojas L, Méndez L & Landrum L. (2014). Componentes volátiles de las hojas de *Pimenta racemosa* var. *racemosa* (Mill.) J.W. Moore (Myrtaceae) de Táchira – Venezuela. BLACPMA. 13 (3): 305 – 310.
52. Kikuzaki H, Miyajima Y, Nakatani N. *Phenolic glycosides from berries of Pimenta dioica.* Journal of natural products, 2008; 71(5): 861-865.
53. González TL, Rodriguez LP. *Estudio fitoquímico preliminar de las hojas y tallos de la especie Pedillanthus tithymaloides (L). Poit.* Revista Cubana de Química, 2010; 15(3): 14-17.
sus propiedades biomedicinales. Revista Horizonte Médico| Volumen, 2007; 7(1): 23.
54. Dupont G, Dulou R, Clément M, et al. *Mémoiresprésentés à la sociétéchimique: Études de l'huileessentielle de Bay de Porto-Rico.* Bull. Soc. Chim. Fr 1954 ; 227: 1082-1084.
55. Abaul J, Bourgeois P, Bessiere JM. *Chemical composition of the essential oils of chemotypes of Pimenta racemosa var. racemosa (P. miller) J. W. Moore (Bois d' Inde) of Guadeloupe (F.W.I.).* Flavour Frag J. 1995; 10(5): 319-21.
56. Bello A, Rodriguez ML, Castineira N, et al. *Chemical composition of the leaf oil of Pimentaracemosa (Mill.) J. Moore from Western Cuba.* J EssentOil Res. 1995; 7(4): 423-424.
57. Ayedoun AM, Adeoti BS, Setondji J, et al. *Aromatic plants from tropical West Africa. IV. Chemical composition of leaf oil of Pimenta racemosa (Miller) J.W. Moore var. racemosa from Benin.* J EssentOil Res. 1996; 8(2): 207-209.
58. Bello A, Urquiola A, García JJ, et al. *Essential oil from leaves of Pimenta racemosa (Mill) J. Moore (Myrtaceae) from Western Cuba.* Ing.Cienc.Quim. 1998; 18(1): 21-23.
59. Chapron N, Parfait A, Bourgeois P. *Industrial importance of essential oils of "Bois d' Inde" Pimenta racemosa var. racemosa. (M. Miller) J.W. Moore.* RivistaItal. EPPOS. 1998; (Numero Speciale): 467-474.
60. Junheon K, Yeon-Suk L, Sang-Gil L, et al. *Fumigant antifungal activity of plant essential oils and components from West Indian bay (Pimenta racemosa) and thyme (Thymus vulgaris) oils against two phytopathogenic fungi*. Flavour Fragr. J. 2008; 23: 272-277
61. Ogundajo A, Owolabi, MS, Oladosu IA, et al. *Volatile Constituents and Potatoes Tuber Sprout Suppressant Activity of Pimentaracemosa (Mill) J.W. Moore.* African J. Basic & Appl. Sci. 2011; 3(3): 92-97.
62. Alitonou GA, Noudogbessi JP, Sessou P, et al. *Chemical composition and biological activities of essential oils of Pimenta racemosa (Mill.) JW Moore. from Benin.* Int. J. Biosci. 2012; 2(9): 1-12.
63. Pragadheesh VS, Yadav A, Singh SC. *Leaf Essential Oil of Cultivated Pimenta racemosa (Mill.) J.W. Moore from North India: Distribution of Phenylpropanoids and Chiral Terpenoids*. Med Aromat Plants 2013; 2(1): 118-121.

Table 1. Phytochemical screening of alcoholic extracts achieved from different parts of *P. racemosa* var. *racemosa.*

Secondary metabolite	Testing	L	F	TS	TSt	C	R
Alkaloids	Wagner, Mayer, Dragendorff	-	-	-	-	-	-
Coumarins	Fluorescence at 365nm	-	-	-	-	-	+
Glycosides	Reaction with $NaOH_{(ac)}$	-	+	-	-	-	-
Glycosides cardiotonic	Keller – Killiani (deoxy glycosides nuclei)	+++	++	+	+	++	+
	Reaction of Legal (cardenolides, or α,β–unsaturated lactones nuclei)	++	+	+++	+++	++	+++
Flavonoids	Shinoda (aurones, chalconnes, flavones, flavonols and flavones)	-	-	-	-	-	-
	Pew´s (dihyflavones, flavonones, and dihydrochalcones)	-	-	+	-	++	+
	NaOH 10% (xanthones and/or flavones, flavonols, chalcones and anthocyanins)	++	++	+	++	++	++
	TLC: 2-aminoetil difenil Borato	++	+++	-	-	-	-
Steroids y/o Terpenoids	Lieberman Bouchard	+++	+	+++	++	++	+++
	Rosenthaler (Terpenoids)	+++	+	+++	+++	+	++
	Salkowskí (Steroids)	+++	+	+++	+++	+++	+++
	TLC: Komarowsky	+++	+	++	++	+++	++
Phenols	$FeCl_3$ 10%	+++	+++	+++	+++	+++	+++
Quinones	$C_6H_{6\ (conc.)}$ (anthraquinone)	-	-	-	-	-	-
	$NH_4OH_{\ (conc.)}$ (anthraquinone)	-	-	++	++	++	++
	Borntraguer (quinone or anthrones derivatives)	-	-	-	-	-	-
	$H_2SO_{4\ (conc.)}$ (quinone)	+	++	++	+++	++	++
Saponins	Foam height	+	-	+	++	+	+++
	Foam (with sodium bicarbonate)	+	-	++	+	+++	+++
Tannins	1% Gelatin, Gelatin-Salt solution (1% gelatin + 10% salt), Potassium ferricyanide 1%	-	-	-	-	-	-
	$FeCl_3$ 10% (catechol or pyrogallol nuclei)	+++	+++	+++	+++	+++	+++

Key: - Absence, + Poor, ++ Moderate, +++ Abundant, L: alcoholic extract of leaves, F: alcoholic extract of fruits, TS: alcoholic extract of fine branches, TSt: alcoholic extract of large branches, C: alcoholic extract of cortex and R: alcoholic extract of roots.