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DETERMINATION OF THE METABOLITES OF RUDA LEAF (*RUTA GRAVEOLENS* L.) CULTIVATED IN CAMPECHE

Marvel, del Carmen Valencia Gutiérrez
``Universidad Autonoma de Campeche``.
ORCID 0000-0002-3671-0296.

Magnolia del Rosario López Méndez
``Universidad Autonoma de Campeche``.
ORCID: 0000- 0002- 7919- 894X.

Suemi Guadalupe del Rosario Can Tun
``Universidad Autonoma de Campeche``.
ORCID: 0000- 0002-6058-9799.

Ivone Yanete Huchim Cahuich
``Universidad Autonoma de Campeche``.
ORCID: 0009-0007-4965-0783.

Noelia Candelaria Fabian Ek
``Universidad Autonoma de Campeche``.
ORCID: 0009-0007-7196-915X

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Abstract: Rue (*Ruta graveolens* L.) is a plant that is found all year round in the State of Campeche, its leaves are used in the Chenes region and in Champotón for headaches, headaches, or rashes. The results obtained from drying in an oven, in aqueous extract at room temperature are: lactones +, alkaloids +, essential oils +, in hot aqueous extract are: alkaloids +, essential oils +, cardiotonic glycosides +, reducing sugars +; in ethanolic extract at room temperature they are: quinones +, flavonoids +, alkaloids +, essential oils +, reducing sugars +; in the hot aqueous extracts they are: phenols +, tannins +, quinones +, flavonoids +, alkaloids +, essential oils +, reducing sugars +. The presence of flavonoids gives it antioxidant action, the primary amino acids share the ability to polymerize with each other to form proteins. With this information, it is achieved that this resource is valued and that those who have it in their yard keep it for the benefits it offers.
Keywords: Rue, Screening, Secondary Metabolites

INTRODUCTION

Ruda (*Ruta graveolens* L.), belongs to the Rutaceae family, it is a native plant of southern Europe and Asia minor origin, it is an evergreen shrub corresponding to the order Rutales, it has approximately more than 1600 species with 150 genera scattered mainly in temperate and tropical zones. of the entire globe (Montero, 2021). Of the existing species of rue, two are found mainly in America; the ruta graveolens and the ruta chalepensis. Both entered the continent through contact with the Spanish and, therefore, hand in hand with the herb, also entered the European beliefs about the magical powers of rue and the knowledge of its therapeutic actions (Forero, 2020).

It is currently naturalized and cultivated in various parts of the world. In America it is found in Canada, the United States, Mexico, Bolivia, Brazil, Colombia, Ecuador, Peru and Chile. (Gutierrez *et al.*, 2017). It is considered as an ornamental plant, as a home remedy, culinary condiment, and as a protector of "bad vibes". It is a resistant plant, it lives an average of 2 years (Coba, 2022).

Rue is also called arruda, rue, péganeon, ruta, aruga, raute (Cusquipoma, 2018). It adapts easily in climates where there is abundant sunlight or semi-shade, in hot, temperate, or cold climates. But it requires protection from strong winds. Rue is an undemanding plant, therefore it adapts to various types of soils, especially limestone and siliceous. It requires watering in a range of 2 times a week, without excess, intensifying watering in summer and suspending it in winter.

It is sown with seeds or by cuttings. The seeds germinate in 2-3 weeks and are then transplanted. The cuttings are placed in rows at a distance of 70 cm between rows and 40 cm between plants. It is cultivated and pruned after flowering to encourage firm and renewed growth. It is fertilized with manure or compost or calcium triple superphosphate. The flowering season is in the months of May-August, therefore, they are harvested in the bud stage, since there it has a greater amount of active ingredients. If leaves and stems are needed, it is harvested before flowering because its active principles are concentrated in the sap, and it is cut at a distance of 12-15 cm from the ground. It renews quite quickly, therefore they are harvested several times a year. To prepare the sample to work, only the leaves that are in good condition are selected, those with molds or poor condition are eliminated (Cusquipoma, 2018).

DEVELOPMENT

One of the ways to publicize the benefits and properties of natural resources is through the study known as Phytochemical

Screening that is carried out in a laboratory, to determine the metabolites present, and allows us to understand why rue is not only used as ornament, but rather its various uses are through the empirical knowledge of the elderly, who use the leaf for its properties, and in this case it is scientifically based.

METHODOLOGY

To carry out the determination of the metabolites, the techniques of the works carried out by the Ministry of Public Health (MINSAP) were used.

To carry out this work, rue leaves collected in Pomuch, Hecelchakán, Campeche were used, the base raw material was the leaves of the rue plant.

Figure 1 shows the diagram of the process used during the development of this work, where it is observed that the initial sample before drying corresponds to 600 g per sample, from which fractions of 10 g were subsequently taken for each type of extract, whether aqueous or ethanolic, at room temperature (RT * or hot, respectively.

The techniques for carrying out phytochemical screening are mentioned below, they were elaborated based on the work carried out by the Ministry of Public Health (MINSAP, 1997).

Ferric chloride technique (phenols and tannins).

Bornträger test (Quinones).

Shinoda method (Flavonoids).

Baljet test (Lactones).

Dragendorff method (Alkaloids).

Sudan Test (Essential Oils).

Ninhydrin technique (free amino acids or amines).

Determination of triterpenes. Libermann -Burchard test (Steroids and triterpenes).

Kedde test (cardiotonic glycosides).

Determination of Anthocyanhydrins.

Fehling test (reducing sugars).

RESULTS

The results obtained from the determination of the Phytochemical Screening of rue (*Ruta graveolens* L.), in the extraction solvents: water and ethanol, used at room temperature and hot as the case may be.

The presence of Phenols was found, these are the main secondary metabolites of plants and their presence in the animal kingdom is due to their ingestion. Phenols are synthesized de novo by plants and are genetically regulated, both qualitatively and quantitatively, although environmental factors also exist at this level. In addition, they act as phytoalexins (injured plants secrete phenols to defend themselves against possible fungal or bacterial attacks) and contribute to the pigmentation of many parts of the plant (for example, anthocyanins are responsible for the red, orange, blue, purple color or violet that we find in the skins of fruits and vegetables). On the other hand, when phenols are oxidized, they give rise to quinones that give a brown color that is often undesirable.

In high concentrations it is very toxic. It causes kidney irritation and even death, but its ingestion is unlikely due to its unpleasant taste. It is toxic to fish. Widely used as a bactericide, but it is biodegradable.

The presence of Tannins was found, which stand out among the natural bioactive compounds for their marked antioxidant and antimicrobial activity (Balasundram *et al.*, 2006; Daglia, 2012). Tannins are phenolic compounds soluble in water and in other substances (alkalis, alcohol and acetone) forming colloidal solutions (Akiyama, 2001). They present molecular weights between 500 and 3,000 daltons (Porras-Loaiza *et al.*, 2009) and a structure with a polyphenolic character (12-16 phenolic groups and 5-7 aromatic

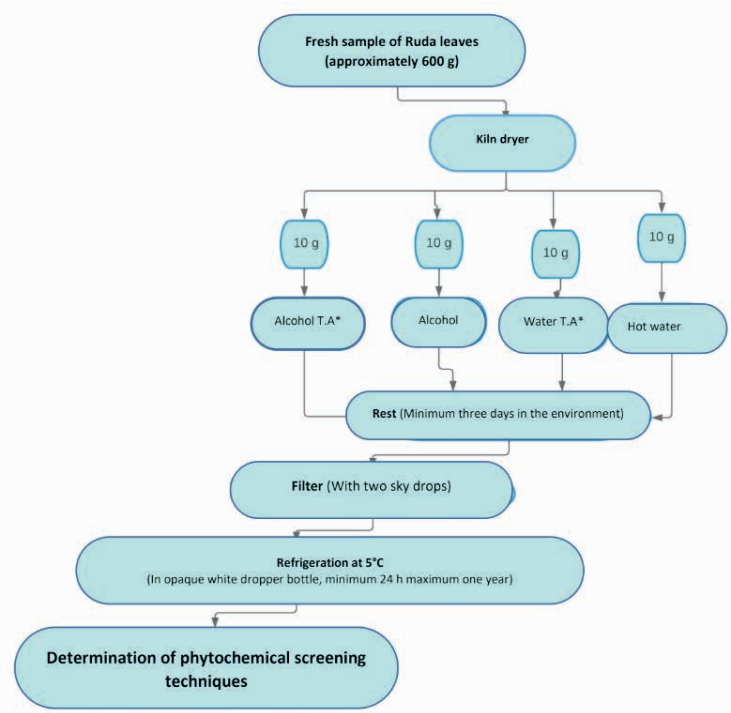

Figure 1. Process diagram

Source: the own author

Metabolites	Aqueous Extract Room temperature	Aqueous Extract Hot	Ethanolic Extract Room temperature	Extract ethanolic Hot
Phenols	+	+	-	-
Tannins	+	+	-	-
Quinones	-	-	-	-
Flavonoids	+	+	+	+
Lactones	+	+	-	-
Alkaloids	+	+	-	-
Essential oils	-	-	+	+
Amino acids	+	+	+	+
Triterpenes	-	-	+	+
Cardiotonic glycosides	-	-	-	-
Anthocyani dins	-	-	-	-
Reducing sugars	+	-	+	+

Table 1 Results of Phytochemical Screening of the Ruda leaf (*Ruta graveolens* L.)

Source: the own author

rings per 1,000 mass units), and can be found in areas of the plant such as stems, wood, leaves and seeds (Isaza, 2007). They have the ability to associate with divalent and trivalent ions and precipitate salts, such as heavy metal salts, Fe3+, Pb, Zn, Cu. In addition, they have a beneficial effect on human health (Vasquez, 2020), attributed to their antioxidant and anticancer activity (Balasundram *et al.*, 2006). According to Freudenberg, tannins are classified based on their structure. Depending on whether the polymerization is between the elemental molecules or between others, they are differentiated: hydrolyzable or pyrogallic tannins, condensed tannins or pyrocatechins and phlorotannins (Álvarez, and Lock de Ugaz, 1992). Hydrolyzable tannins are esters of phenolic acids in particular gallic or ellagic acid, and simple sugars. They are smaller than condensed tannins and are easily hydrolyzed in a dilute acid medium. With respect to condensed tannins (sometimes also called proanthocyanidins), they are compounds derived from flavan-3,4-diol units joined together by CC bonds in positions 4-8 and 4-6 (Isaza, 2007; Álvarez and Ugaz's Lock, 1992).

Due to the presence of flavonoids in the leaf, these have their known antioxidant capacity, and flavonoids are used to treat diseases related to inflammatory processes and cardiovascular disorders, because they improve peripheral circulation, cholesterol mobilization and decrease capillary fragility. Its hepatoprotective, antiallergic, antithrombotic, anticancer, antibacterial, and antifungal activity, among others, has also been documented (Jiménez *et al.*, 2003).

Lactones were present, the interest in these molecules is justified by the multiple biological activities they present, among which the antineoplastic and cytotoxic activity stand out, both linked to the function of the α-methylene-γ-lactone grouping, apparently by nucleophilic attack of certain active centers of the proteins to the double bond through a Michael addition. Thus, the thiol groups of cysteine appear to be the primary targets of sesquiterpene lactones, which result in the inhibition of various cellular functions leading to cell apoptosis. In essence, the interaction between them and the thiol groups of the proteins causes a reduction in the enzymatic activity or causes the interruption of the metabolism, which is of vital importance in the intracellular redox balance of the cell.

The presence of Alkaloids provides properties to the leaves since they constitute a highly varied group, both taxonomically and chemically, being a basic nitrogen the only unifying factor of the different classes. For this reason, questions of the physiological function of alkaloids in the plant, their importance in taxonomy, and biogenesis are often more discussed at the level of a particular class of alkaloid, a similar situation with the therapeutic and pharmacological activities of the alkaloids. Since most alkaloids in medicine are herbal, they have always been important in the allopathic system, where the dosage is strictly controlled, and in homeopathy, where the dosage rate is as low as harmless (Evans, 2009).

On the other hand, essential oils are aromatic oily liquids that are obtained by different extraction methods, from plant material (flowers, stems, roots, leaves, fruits, and seeds), some of them indicate antibacterial and antifungal activity, evaluated as a potential source of new antimicrobial compounds and an alternative for food preservation. Essential oils are complex mixtures of up to more than 100 components that can be low molecular weight aliphatic compounds (Fernández, 2022).

Essential oils in Ruda Graveolens: Composed of esters (2-nonyl and 2-undeyl acetates, etc.); methylnonyl, methylheptylketone; monoterpenes (α and β-pinene, limonene),

aliphatic ketones (methylnonylketone in a proportion of 90%), alcohols (2-undecanol), coumarins and furanocoumarins (0.15-0.70%) highlighting: bergapten, psoralen, daphnoretin, isoimperatorin, scopoletin, umbelliferone, pangelin, etc. (Quishpe, 2018).

The amino acids in the leaves provide you with the multiple functions that they fulfill in the organism, especially with regard to the construction and regeneration of tissues. Lysine, for example, has been shown to help control herpes simplex, while arginine has been shown to have remarkable palliative qualities in cases of rheumatoid arthritis. Tryptophan is important because it is the raw material from which the body manufactures the neurotransmitter serotonin and also the hormone melatonin, which promotes sleep and is a powerful antioxidant. Enzymes help carry out the chemical reaction of vitamins, minerals and hormones.

Terpenes or terpenoids are compounds that are built from isoprene units, so their structures can be divided into five carbon (C5) units (Hanson, 2003).

Terpenes are synthesized from primary metabolites by two pathways: the mevalonic acid (MEV) pathway, which occurs in the cytosol, and the methylerythritol phosphate (MEP) pathway, which is found in chloroplasts. In the MEV pathway, three molecules of acetyl-CoA condense to form mevalonic acid, which reacts to form isopentyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP), the substrates for the formation of larger terpenes. IPP can also be generated by the MEP route, through the production of 2-Cmethyl-D-erythritol 4-phosphate from pyruvic acid and glyceraldehyde 3-phosphate. IPP and DMAPP are the activated precursors in terpene biosynthesis in condensation reactions catalyzed by Prenyl Transferases to give rise to prenyl. biphosphates such as geranyl diphosphate (GPP), a monoterpene precursor, farnesyl diphosphate (FPP) a sesquiterpene precursor, and geranylgeranyl diphosphate (GGPP) a diterpene precursor.

The presence of reducing sugars gives the leaves the functions of acting since its components are rich in cellulose, therefore, they are considered to be a potential source of sugars.

CONCLUSIONS

Finally, the presence of Flavonoids and Amino Acids in the rue leaves was found in all the alcoholic extracts, as well as in the aqueous extracts at room temperature and at hot temperature. The presence of Flavonoids provides the pharmacological action to treat diseases related to inflammatory processes and cardiovascular disorders, because they improve peripheral circulation, cholesterol mobilization and decrease capillary fragility. The functioning of amino acids and enzymes is fully interactive, thus, the insufficiency or lack of one of them negatively affects the functioning of the entire system. These pharmacological benefits are what the people of Campeche take advantage of to use, since it is used as a remedy by grandmothers to treat a variety of ailments in the family. This allows awareness about the cultivation, use and conservation of the resource for the benefits it offers, it can be planted in the patio of their houses or in pots, it helps the household economy because it is used and marketed and most importantly it is transmits the herbal knowledge of the elderly to the new generations based on scientific studies.

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