SCREENING OF THE BIOLOGICAL ACTIVITY FROM ESSENTIAL OILS OF NATIVE SPECIES FROM THE ATLANTIC RAIN FOREST (SÃO PAULO – BRAZIL)

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Summary

The essential oils from *Symphyopappus itatiayensis* (Asteraceae), *Myrciaria floribunda* (Myrtaceae), *Talauma ovata* (Magnoliaceae), *Psidium cattleyanum* (Myrtaceae) and *Nectandra megapotamica* (Lauraceae) were assayed for antimicrobial, anti-inflammatory and antitumor activities. All the oils tested were active towards *S. aureus* and *P.aeruginosa* and none of them could be considered active against *E. coli*. The oil of *P. cattleyanum* was the only one which significantly inhibited the growth of *C. albicans* (42%). In the anti-inflammatory assay, only oils active were those from *P. cattleyanum* and *N. megapotamica* and three of them caused toxic effects to the leukocytes, *M. floribunda, S. itatiayensis* and *T. ovata*. The antitumor assay showed that only the oils of *N. megapotamica* and *M. floribunda* were able to cause lethality higher than 50% to at least one of the cell line. The results indicated that the essential oils have a pharmacological potential as antimicrobial, anti-inflammatory and antitumoral agents.

Key Words: *Symphyopappus itatiayensis, Myrciaria floribunda, Talauma ovata, Psidium cattleyanum, Nectandra megapotamica,* essential oils, antimicrobial, anti-inflammatory, antitumor.

Introduction

The traditional medicine all over the world employs aromatic plants in the treatment of microbial infections and as anti-inflammatory. The anti-septic properties attributed to the essential oils are the most recognized, being already mentioned in the Bible and were employed by the ancient Egyptians as embalming ointments during the mummification procedure. The aromatic properties of the plants are conferred by the presence of volatile oils. These oils consist of a mixture of low molecular weight substances, mainly mono and sesquiterpenes, and are found almost exclusively in the spermatophyte group. The genuses which are able to synthesize this class of metabolites are especially found in the following dicotyledonous angiosperm families: Lauraceae, Myrtaceae, Rutaceae, Asteraceae, Rosaceae, Pinaceae, Apiaceae, Myristicaceae, Lamiaceae and Verbenaceae.

The plant variety in tropical forests represents a rich source for searching new bioactive volatile compounds. Brazil has one of the largest plant biodiversity in the world, distributed in different biomes. Only in the State of São Paulo, there are two important biomes, the Atlantic rain forest and the Cerrado, considered as hotspots for conservation priority. The Atlantic Forest in São Paulo is characterized by a great diversity of Lauraceae and Myrtaceae species (1), considered as important producers of essential oils.

Nowadays, scientific research has demonstrated that the volatile oils present a myriad of pharmacological activities such as, analgesic, antimicrobial, antimalarial, antitumor, anti-inflammatory and cardiovascular, activities besides action on the central nervous system and gastro-protector. The anti-septic power depends on the nature of the active constituents found in the oils. In principle, the most active are those rich in phenols, as thymol, carvacrol, eugenol and isoeugenol, alcohols and ketones, such as menthol, menthone, terpinen-4-ol, α and β-pineno (2).

In order to access the biological potential of the aromatic plants from the Brazilian Atlantic Forest, we assayed the essential oils from *Symphyopappus itatiayensis* (Asteraceae), *Myrciaria floribundus* (Myrtaceae), *Talauma ovata* (Magnoliaceae), *Psidium cattleyanum* (Myrtaceae) and *Nectandra megapotamica* (Lauraceae) for antimicrobial, anti-inflammatory and antitumor activities.

Methods

Plant material: The plants were collected from State Park Ilha do Cardoso, in Cananéia – SP, and from Experimental Station Fazenda Campininha, in Mogi-Guaçu-SP. A voucher specimen was deposited at the Herbarium of the Instituto de Botânica de São Paulo (SP).

Essential oil extraction: The essential oils were obtained of dried leaves by hydrodistillation for 4 h in a Clevenger-type apparatus. The oils were collected, dried over anhydrous sodium sulfate, weighted and then stored at -25^{o}C until testing.

Antimicrobial Assay: The essential oils were tested with Gram-positive model bacteria, *Staphylococcus aureus* subsp. *aureus* (ATCC 25923), two Gram-negative models, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 9027) and yeast *Candida albicans* (ATCC 10231). A broth microdilution method was used to determine the antimicrobial activity (3,4,5). All tests were performed in Tryptose soya broth, with the exception of the yeast which employed Sabouraud dextrose broth. The essential oils were diluted in a proportion of 1:4 with a solution of Methanol: Dimethylsulfoxide 1:1. For the assay, 10 µL of the essential oil dilution was inoculated in 190 µL of an overnight broth culture with the final concentration of the microbe adjusted to 2 x 10^3 CFU/ml in each well. The plates were incubated at 37^{o}C for 24 h for bacteria and at 25^{o}C for 48 h for the yeast. The growth inhibition was determined by reading the absorbance at 630nm (SLT Spectra). The samples were analyzed in duplicate and the assay was repeated twice. The antibiotics Chloramphenicol, Amikacin and Nystatin were employed as positive controls.

Anti-inflammatory Assay: In this assay, the samples of volatile oils prepared as an emulsion with Tween-80 in a ratio of 9:1 and as a control sample water emulsion in Tween-80 (9:1). The neutrophils were obtained from male adult Wistar rats weighting between 180-220 g. The anti-inflammatory activity was evaluated by the Boyden technique (6) modified by Zigmond & Hirsch (7). Cylindrical chambers made of transparent acrylic have been used to make two compartments (0.5 mL of capacity each): the lower compartment is filled with a solution containing the chemotactic factor, and the superior one with the suspension of peritoneal neutrophils. The compartments are separated by a cellulose nitrate filter with 13 mm of diameter and 8 µm. In this way, the migration of the cells through the filter can be evaluated in the gradient of concentration of the chemotactic factor that will be established between the two compartments. The chambers had been prepared in duplicate and incubated at 37º C during 1 hour in a moist atmosphere. Afterwards, the filters have been removed, fixed, stained, diaphanized overnight and mounted as a microscope slide. The distance, measured in micrometers, between the superior plan of the filter and two cells in focus, allowed to evaluate the migratory capacity of the neutrophils. The results had been express as the average $\pm$ standard error and statistically analyzed by the Student "t" test or Analysis of Variance (ANOVA) (8).

Antitumor Assay: Human tumour cell lines (MCF-7 breast adenocarcinoma; KM-12, colon adenocarcinoma; RPMI-8226, multiple myeloma; PC-3, prostate carcimoma; SF-268 glioblastoma and NCI-H460, lung great cells carcinoma) are cultivated in tissue culture bottles with half RPMI-1640 medium supplemented with 5 % of bovine

foetal serum and 1 % of L-glutamine. The bottles were kept in an incubator at 37º C with 5 % of CO_2 and 100% of relative humidity. Both adherent and non-adherent cells are transferred weekly to fresh medium, adherent cells were trypsinized before transferring (9). The cell density measured in a Neubauer chamber using the exclusion method with Trypan blue. The cellular density for the assay varies accordingly to the cell line: MCF-7 (10.000), PC-3 (7.500); KM-12 (15.000); NCI-H460 (7.500); SF-268 (15.000) and RPMI-8226 (20.000). The assay is performed by 400 times dilution of the oil samples in RPMI medium, 10 µL of the oil is diluted in 1990 µL of medium and 100 µL of this solution is applied in 100 µL of the cell suspension. The cells were incubated for 24 hours before the addition of the oils. The oils remain in contact with the cells for 48 hours. After the established time, the "end points" were obtained as previously described (9).

Results and Discussion

The yields of the extracted oils is presented in table 1.The highest yield was obtained for the species *T. ovata*.

The oils of the five species were assayed for antimicrobial activity against Gram-positive and Gram-negative model bacteria and a yeast model. The results of the assay are presented in table 2. All the tested oils were showed a strong inhibitory activity towards *S. aureus* and a moderate inhibition of *P. aeruginosa*. On the other hand, for the second Gram-negative microorganism, *E. coli*, none of the oils could be considered active. The oil of *P. cattleyanum* was the only one which significantly inhibited the growth of *C. albicans* (42%). Gram-negative organisms are known to be slightly less susceptible than gram-positive bacteria to essential oils. A number of oil components have been identified as effective antibacterial, e.g. carvacrol, thymol, eugenol, cinnamaldehyde and cinnamic acid (2, 10). The extent of the sensitivity might vary with the strain and environmental conditions imposed. The broader activity observed for the *P. cattleyanum* oil might be due to the high concentration of pinenes. (11, 12, 13).

Table 1- Relative yields of the essential oils extracted from the leaves of 5 Atlantic Rain Forest plant species.

Plant Material	Voucher No	Yield (%)
S. itatiayensis	Moreno 29	0,6
N. megapotamica	Moreno 54	0.1
T. ovata	Moreno 25	1,0
M. floribundus	Moreno 51	0,6
P. cattleyanum	Moreno 40	0,6

Table 2 - Antimicrobial activity of essential oils from native species from the Atlantic rain forest by the microdilution method.

	% Growth Inhibition			
Sample (Conc. 3.125 µL/mL)	*E. coli*	*S. aureus*	*P. aeruginosa*	*C. albicans*
M. floribunda	-	76.0	38.0	5.0
T. ovata	-	60.0	51.5	-
S. itatiayensis	10.0	50.0	39.0	-
N. megapotamica	20.2	71.0	51.0	-
P. cattleyanum	11.0	55.0	58.0	42.0

The anti-inflammatory activity of the oils was assayed and the results are displayed in table 3. In the leukocyte migration assay, only the oils of *P. cattleyanum and N. megapotamica* could inhibit the migration indicating an anti-inflammatory activity. The essential oils of *M. floribundus, S. itatiayensis,* and *T. ovata* caused some toxic effects to the leukocytes (table 3). The anti-inflammatory activity detected in this assay can be explained by the capacity of some essential oil components in inhibiting prol L-1β protein expression induced by the chemotactic factor, as demonstrated for the *Cinnamomum osmophloeum* oil (Lauraceae) (14). In addition, some monoterpenes are known to reduce NO production/release in lipopolysaccharide-induced macrophages (15) which might explain the activity of the *P. cattleyanum* oil, rich in this class of substances (11).

As in the anti-inflammatory assay some of the essential oils presented a cytotoxic effect, their antitumor potential was evaluated. The relative lethality determined for six human tumour cell lines is presented in table 4. In this assay, only the samples which were able to kill 50% of the tumor cells after 48 h of incubation. The oil from *N. megapotamica* presented a selective cytotoxicity for prostate and multiple myeloma cells lines while that from *M. floribundus* showed a specific toxicity, almost 100%, for the glioblastoma cells. Monoterpenes have multiple pharmacologic effects on mevalonate metabolism; some of these effects may account for their tumor suppressive activity because tumor cells synthesize and accumulate cholesterol faster than normal cells (16). Sesquiterepenes, such as caryophyllene, caryophyllene oxide and α-humulene, are also implicated in the cytotoxicity displayed for several essential oils which might be related to the increase in the concentration of reactive oxygen species by depletion of internal glutathione (17,18).

Table 3- Anti-inflammatory activity from essential oils of native species from the Atlantic rain forest evaluated by the leukocyte migration in the Boyden chamber.

Sample	Average Distance (mm)
Control	119,0 ± 3,5 mm
M. floribunda	Internal alterations
T. ovata	Discrete cytoplasm alterations
S. itatiayensis	Cytotoxic
N. megapotamica	16,2 ± 3,8 mm (active)
P. cattleyanum	17,0 ± 2,5 mm (active)

Table 4- Antitumor activity from essential oils of native species from the Atlantic Rain forest against human tumor cell lines (MCF, mammary adenocarcinoma; KM, colon adenocarcinoma; RPMI, multiple myeloma; PC, prostate carcinoma; SF, glioblastoma and NCI, lung great cells carcinoma).

	Lethality (%)					
Oil Sample*	MCF-7	PC-3	NCI	KM	RPMI	SF
S. itatiayensis	<50	<-50	<50	<50	<50	<50
N. megapotamica	<50	65.5	<50	<50	76.2	<50
T. ovata	<50	<50	<50	<50	<50	<50
M. floribundus	<50	<50	<50	<50	<50	95.6
P. cattleyanum	<50	<50	<50	<50	<50	<50

* Final Sample Concentration in the assay = 0.25 µL/mL

The results suggest that the essential oils are important sources new antimicrobial, anti-inflammatory and anitumor agents. The mechanisms of action of these essential oils extracted from native Atlantic Rain forest species need to be further examined for potential uses as well as their chemical composition. This study open perspectives to find more effective drugs of vegetal origin in the treatment of diseases related with these activities.

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