



# Detection of adulterated copaiba (*Copaifera multijuga* Hayne) oil-resins by refractive index and thin layer chromatography

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**RESUMO:** “Detecção de óleo-resinas de copaíba adulterados através do índice de refração e da cromatografia de camada delgada”. Os índices de refração (IR) de oito amostras de óleo resina de copaíba coletadas para este estudo na RDS Tupé variaram de 1,50284 a 1,50786. As placas de cromatografia de camada delgada (CCD) dos óleo-resinas, reveladas com o reagente anisaldeído-ácido sulfúrico, apresentaram um perfil cromatográfico característico, com manchas em lilás escura com várias manchas menores na parte do  $R_f$  baixo e uma grande mancha arredondada, no  $R_f$  alto. Por outro lado, as doze amostras de óleo de copaíba adquiridas no mercado local apresentaram IR entre 1,48176 a 1,50886, e, as placas de CCD apresentaram como perfil característico geral manchas de cor azul, com manchas superpostas menores no  $R_f$  baixo, manchas superpostas maiores com aparência alongada no  $R_f$  alto e uma mancha arredondada incolor no  $R_f$  intermediário. Dentre os doze óleos adquiridos do mercado, a) três óleo-resinas apresentaram IR e perfil CCD semelhantes aos de amostras de óleo de copaíbas coletadas; b) seis óleos apresentaram o mesmo IR e perfil em CCD do óleo de soja; e, c) três amostras apresentaram IR semelhantes aos da amostra do Tupé, porém com o perfil cromatográfico semelhante à uma mistura de óleo de soja: óleo de copaíba, sendo duas na proporção de 3:1 e uma na proporção de 1:3. Portanto, a determinação do IR e a análise do perfil em CCD podem ser considerados ensaios rápidos e eficientes para a detecção de óleos vegetais em óleo-resina de copaíba.

**Unitermos:** *Copaifera multijuga*, Fabaceae, óleo-resina de copaíba, índice de refração, cromatografia de camada delgada.

**ABSTRACT:** The refractive indices (RI) of the eight samples of copaiba oils, collected for this study at RDS Tupé ranged from 1.50284 to 1.50786. The thin layer chromatography (TLC) plates of these oils revealed with anisaldehyde-sulphuric acid reagent showed dark lilac stains with several small stains at low  $R_f$  and a large rounded stain at high  $R_f$ . On the other hand, the twelve copaiba oils purchased at local markets presented RI between 1.48176 and 1.50886, and the TLC plates, showed as general profile blue stains, with smaller superimposed stains at low  $R_f$ , bigger superimposed stains like elongated stain at high  $R_f$  and a colorless rounded stain at middle  $R_f$ . Among 12 purchased oils at local markets, a) three oil-resins presented similar RI and TLC profile to those observed for collected copaiba oils; b) six oils showed same RI and TLC profiles to those observed for soybean oil; c) three samples presented RI near to those showed by copaiba oil-resin, however the TLC profile was near to profile observed for a prepared mixture soybean oil: copaiba oil, two samples with 3:1 proportion and one sample with 1:3 proportion. Therefore, the RI determination and the TLC profiles could be considered rapid and efficient procedures for detection of vegetal oil in the copaiba oil-resins.

**Keywords:** *Copaifera multijuga*, Fabaceae, copaiba oil-resin, refractive index, thin layer chromatography.

## INTRODUCTION

The copaiba oil-resin is one of the most widely phytomedicine used in Brazil, mainly in the North Region, including Amazon. The copaiba oil-resin is extensively used in traditional medicine as antiinflammatory and cicatrizing drugs, as well as antitetic, antibleorrhagic and antirheumatic (Cascon,

2004; Tavares et al., 2006; Agra et al., 2007, 2008).

These traditional uses came after indian observation, that injured animals rubbed themselves against the barks of the copaiba trees for the treatment. Thus, the first use of copaiba oil was for treatment of healings and inflammations (Cascon, 2004). Some fractions of *Copaifera multijuga* oil-resin present anticancer activity *in vitro* and *in vivo* assays (Lima et

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al., 2003), and antibacterial activity (Vasconcelos et al., 2008).

The copaiba oil-resin is a product obtained by artisanal procedures. The low availability of this oil in local markets, compelled to the adulteration, since the beginning of the last century, in order to supply the high demand of this product from European and Brazilian markets (Veiga Jr & Pinto, 2002).

The adulteration of the copaiba oil-resin commercialized in Brazil is made, according to literature (Cascon, 2004), under two modes: a) adding vegetal oil, such as soybean oil, or mineral oil; and b) adding other vegetal oils with a similar density and flavor, but from species of another genera or family (Tappin et al., 2004).

Frequently, it occurs also collection of oil-resin of different species of *Copaifera*, because there are seven different species of *Copaifera* in the Amazon Rain Forest. These oil-resins present different chemical compositions, but this case is not considered adulterated copaiba oil-resins (Veiga Jr & Pinto, 2002).

Many techniques for quality control of copaiba oil-resin can be found in the literature. The High Resolution Gas Chromatography (HRGC) shows the occurrence of two groups of compounds in authentic oil-resin used as a reference and it was observed absence of compounds belonging to the volatile group in the commercial samples indicating an adulteration (Tappin et al., 2004).

Some indices, as saponification and acidity values, were investigated for quality evaluation, and it was established that an acidity value above 80 mg KOH/100 g oil-resin indicates an adulteration (Vasconcelos & Godinho, 2002).

The quantification of the sesqui- and diterpenes in commercial oil-resins were also considered in order to detect the adulteration of the samples (Veiga Jr et al., 1997; Biavatti et al., 2006).

This study intends to detect adulterations in the commercial samples of copaiba oil-resin by use of the TLC and refractive index, avoiding undesirable effects, because this important product from the Amazon biodiversity it is frequently used in Brazilian folk medicine for disease treatments.

## MATERIAL AND METHODS

Eight reference oil-resins (C.01, C.02, C.03, C.04, C.05, C.06, C.07, C.08) were collected according to Leite et al. (2002) by perforation of trunk wood of the *Copaifera multijuga* Hayne specimens (Figure 1), during the rainy and dry (C.08) seasons at the Reserva de Desenvolvimento Sustentável do Tupé (RDS-Tupé), situated on the left bank of the Rio Negro, 25 km far from Manaus-AM (Figure 3).

Twelve commercial copaiba oils (P.01 to P.12) were purchased from Mercado Municipal Adolpho Lisboa and from other markets from Manaus-AM. The

origins of the samples are showed in Table 1.

The mixtures of soybean oil and copaiba oil-resin (C.01) were prepared in proportions of 3:1; 1:1 and 1:3, respectively.

The refractive indices of these oils were determined on an Abbé type refractometer, manufactured by Optronics Inc., performed in duplicate readings at 20 °C.

The chromatographic profiles of the 24 samples were observed on TLC plates (SiO<sub>2</sub>, 60, F254) manufactured by Merck. The TLC plates were eluted with hexane:ethyl acetate 9:1 solvent system. The eluted plates were revealed spraying anisaldehyde-sulphuric acid reagent followed by heating at 120 °C.

## RESULTS AND DISCUSSION

The refractive index of the collected copaiba oils ranged between 1.50184 and 1.50786 (Table 2) at 20 °C. These values are in according to described value of 1.5065 at 24 °C (Gottlieb & Iachan, 1945).

The TLC plates of these collected oils revealed with anisaldehyde-sulphuric acid reagent showed dark lilac stains with several small stains at low R<sub>f</sub> and a large rounded stain at high R<sub>f</sub>. The TLC plates of C. 01 and C.04 samples are shown in Figure 3a.

The refractive indices corresponding to the commercial oil-resin samples varied from 1.48176 to 1.50886 (Table 3).

Among twelve commercial samples analyzed, three samples (P.02, P.06, and P.09) showed refractive indices and TLC profiles similar to those observed for the reference oil-resin from RDS-Tupé.

Six commercial samples (P.01, P.03, P.04, P.05, P.07, P.11) presented refractive indices close to those found for soybean oil: 1.47473. These values agree with those described in the literature which is 1.4737 (Falate et al., 2007).

TLC plates, showed as general profile blue stains, with smaller superimposed stains in the low R<sub>f</sub>, bigger superimposed stains with shape of elongated stain in the high R<sub>f</sub> and a colorless rounded stain in the middle R<sub>f</sub>. In the Figure 3a are shown TLC plates of samples P.01 and P.04.

The refractive indices of soybean oil and collected oil-resin mixtures, respectively in 3:1, 1:1 and 1:3 proportions are show in Table 4.

The TLC profile of soybean oil presents a large light rounded stain in the middle R<sub>f</sub> and an elongated stain in the high R<sub>f</sub>; the 3:1 proportion presented small blue stains in the low R<sub>f</sub>, a large colorless rounded stain in the middle R<sub>f</sub> an elongated stain in the high R<sub>f</sub>; the 1:1 and 1:3 proportions presented similar profiles to the 3:1 proportion, but different in size of the stains (Figure 3b).

The commercial samples (P.08, P.10 and P.12) presented refractive indices similar to those found in the collected samples; however the chromatography

**Table 1.** Origins and colors of the oil-resins purchased from Manaus-AM markets.

Samples	Origin	Color
P.01	Amazon Inland	Light yellow
P.02	Janauacá-AM	Light yellow
P.03	Manaquiri-AM	Light yellow
P.04	Lábrea-AM	Dark yellow
P.05	Lábrea-AM	Light yellow
P.06	Santarém-PA	Light yellow
P.07	Altazes-AM	Light yellow
P.08	Rio Purus AM	Dark yellow
P.09	Lábrea-AM	Light yellow
P.10	Amazon Inland	Light yellow
P.11	Parintins-AM	Light yellow
P.12	Rio Purus AM	Light yellow

**Table 2.** Refractive indices ( $\eta_D$ ) at 20 °C of oil-resins from *C. multijuga* collected at the Reserva de Desenvolvimento Sustentável do Tupé, Manaus-AM.

Reference Samples	$\eta_{D_{20}}$
C.01	1.50284
C.02	1.50585
C.03	1.50284
C.04	1.50786
C.05	1.50585
C.06	1.50585
C.07	1.50585
C.08	1.50385

**Table 3.** Refractive indices ( $\eta_D$ ) at 20 °C of samples purchased in markets Manaus-AM.

Samples	$\eta_{D_{20}}$
P.01	1.48477
P.02	1.50385
P.03	1.48176
P.04	1.49180
P.05	1.48578
P.06	1.50385
P.07	1.47875
P.08	1.50585
P.09	1.50284
P.10	1.50886
P.11	1.48579
P.12	1.50485

**Table 4.** Refractive indices at 20 °C of mixtures of soybean oil (S.O.) and copaiba oil resin (C.01) collected at the Reserva de Desenvolvimento Sustentável do Tupé, Manaus-AM.

Proportions	$\eta_{D_{20}}$
3:1	1.48477
1:1	1.49180
1:3	1.50183


**Figure 1.** Perforation of *Copaifera multijuga* tree for oil-resin collection at the Reserva de Desenvolvimento Sustentável do Tupé, Manaus-AM.

**Figure 2.** Location of the Reserva de Desenvolvimento Sustentável do Tupé, Manaus-AM.

profiles of P.08 presented similar to 3:1 proportion TLC profile and the P.10 and P.12 samples presented similar profiles to those observed in the 1:3 proportion profile.

According to Shriner (1997), TLC is considered the most useful technique for assessing the purity of organic compounds and Simões & Sptizer (2004) add that the TLC plate is characteristic for each oil and it allows to confirming to detect fake oils.

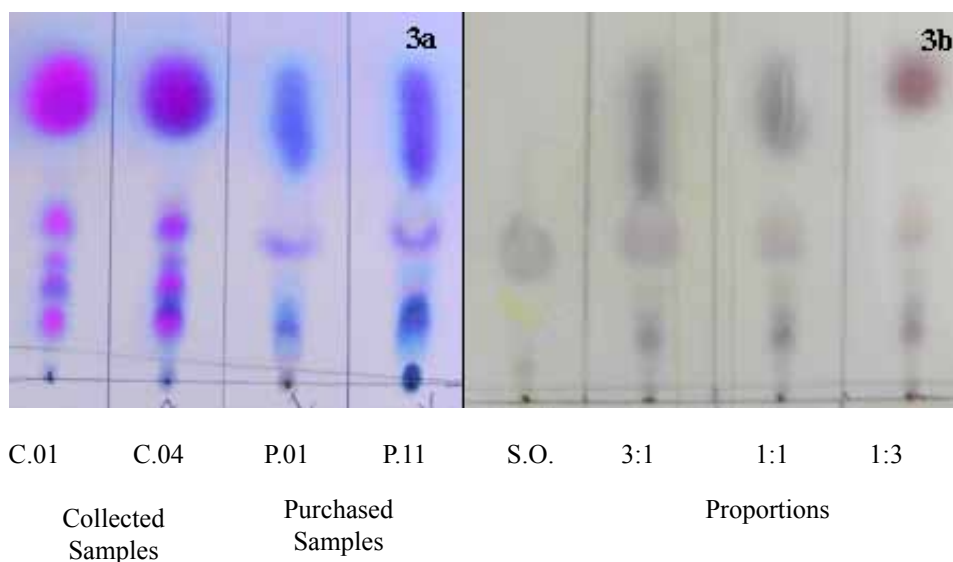
TLC as quality control of copaiba oil resin can be easily employed in herbalist laboratories, pharmacies, industries, universities, and etc.

## CONCLUSION

The technique developed to detect adulteration of copaiba oil-resin led to conclusion that the association of refractive indices and thin layer chromatography is efficient, rapid and low cost analyses for product quality control.

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**Figure 3.** TLC profiles of oils on Silica 60, F254 plates eluted with hexane:ethyl acetate 9:1, revealed with anisaldehyde-sulphuric acid reagent. **3a.** TLC plates of collected samples (C.01 and C.04) and purchased samples (P.01 and P.04). **3b.** TLC profile of the samples: soybean oil (SO) and 3:1, 1:1 and 1:3 proportions of S.O.: C.01.

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