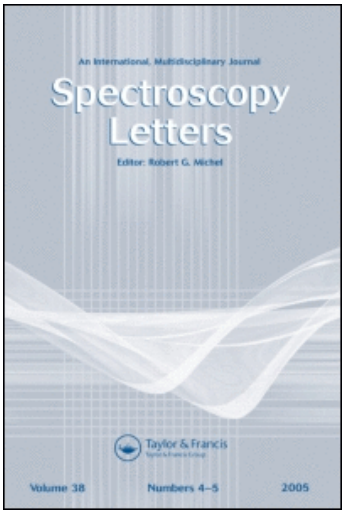

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COLORIMETRIC DETERMINATION OF CRUDE POWDERED MYRRH, PURIFIED MYRRH EXTRACT, OILY FRACTION, AND ITS DIFFERENT PHARMACEUTICAL DOSAGE FORMS

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COLORIMETRIC DETERMINATION OF CRUDE POWDERED MYRRH, PURIFIED MYRRH EXTRACT, OILY FRACTION, AND ITS DIFFERENT PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Myrrh is an effective anti-microbial agent that is considered an excellent external remedy for mouth, throat and skin infections and it is also useful in systematic treatment of glandular fever and brucellosis.

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The purified myrrh extract has been formulated as soft gelatin capsules, suppositories and emulsion known commercially as Mirazid. Recently, Mirazid has been proved to be a safe drug of potential effects as antibilharzial and fasciolicidal agent. In this study, a simple, precise and accurate, colorimetric method for determination of crude powdered myrrh, petroleum ether extract (oily fraction) and purified myrrh extract, in soft gelatin capsules, emulsion and in suppositories, is put forward. The procedure involves reaction with vanillin-sulfuric acid reagent to form a violet colored reaction product with maximum absorption at 518 nm. Optimum conditions for color formation are reported and the color has been found to be stable for six hours. Beer's law is obeyed in a concentration range 40–200 μg/mL, with correlation coefficient of 0.9994. The mean percentage recoveries have been found to be 101.31 ± 0.54 for soft gelatin capsule, 100.18 ± 0.39 for suppositories and 100.14 ± 0.41 for emulsion. No interference has been observed either from capsule shell, suppository base or emulsifying agent.

In comparison to a another colorimetric method based on the use of blue tetrazolium salt, the proposed method is more selective, in addition of being simple, of good accuracy, and high precision for determination of the major bioactive components of the oily fraction rather than resin extract. The procedure has been validated for use in quality control applications.

Key Words: Myrrh; Purified myrrh extract; Vanillin-sulfuric-acid; Colorimetry; Pharmaceutical preparations

INTRODUCTION

Myrrh, the oleo-gum resin of *Chommiphora molmol* (family: Burseraceae) is one of the oldest known medicine that was widely used by the ancient Egyptians. It is an effective anti-microbial agent that has been used traditionally either externally, in the treatment of mouth ulcers, gingivitis and sinusitis, or systematically in the treatment of glandular fever as well as brucellosis.

Myrrh consists of a mixture of volatile oil "myrrhol", about 2%–8%; resin "myrrhin", about 23–40%; the remainder of the drug is consisting of gum, about 40–60%[1–3]. Constituents of the volatile oil, myrrhol, include

terpenes such as elemol, guaiol, and α-copaene-8-ol; sesquiterpenes such as curzerenone and lindestreen; esters; cuminic aldehyde and eugenol. The resin, myrrhin, is not entirely soluble in ether. The smaller ether- insoluble portion contains α- and β- heerabomyrrholic acids and the larger ether-soluble portion contains three free resin acids, namely α-,β- and δ-commiphoric acids, esters of a resin acid, commiphorinic acid, and two phenolic resins, α- and β- heerabomyrrhol . The gum is composed of arabinose, galactose, xylose and an oxidase enzyme the activity of which is destroyed by a temperature.

Purified myrrh extract has been prepared in our laboratory by extraction of crude powdered myrrh with methyl alcohol (Method "a"), or by combination between oily fraction and resin (Method "b"), the purified myrrh extract has been formulated as soft gelatin capsules, suppositories and as emulsion known commerically as "Mirazid". The latter is considered as a new natural safe drug of potential effects as antibilharzial agent that has been proved effective against *Schistosoma mansoni* and *Schistosoma haematopium*[4,5]. Evaluation of the molluscicidal activity of Mirazid emulsion of the oily fraction and resin extract of myrrh against the snail "*Biomphalaria alexandrina*", intermediate host of *Schistosoma mansoni* , has proved that this activity is due to the oily fraction rather than the resin extract[5]. In addition , recent study, has proved that Mirazid is a potent fasciolicidal drug with a cure rate of 94.1%[6].

Screening of the literature revealed that only few analytical techniques have been developed for the investigation of the major constituents of the extracts of myrrh, these include analytical supercritical fluid extraction- gas chromatography[7–9], or ^{13}C-NMR and ^{1}H-NMR[3]. Only one colorimertric method has been reported for determination of steroidal content of *Commiphora mukul* and its formulations by blue tetrazolium[10].

During the preparation of the purified myrrh extract from the crude drug, numerous chromogenic detection reagents for sterols, steroids,... have been applied (as spray reagents) to the different chromatographic fractions. Among these chromogenic reagents tested, vanillin-sulfuric acid reagent has been observed to react with both the purified myrrh extract, the crude and the oily fraction to give a violet color. The latter has been found to be stable for over 6 hr and these findings encouraged us to study the quantitative aspects of this reaction and optimization of the different variables for determination of the purified myrrh extract in different available formulations.

Vanillin in strongly acid medium has been widely used for identification and determination of different classes of compounds and natural products. The reagent as a 1% w/v solution in absolute ethanol, and in presence of 80% v/v sulfuric acid has been used for determination of

glycyrrhizic acid in liquorice extract[11]. The colorimetric determination of Ginseng sapogenins (panaxadiol and panaxatriol) or their corresponding saponins (ginsenosides Rb_1, Rd and Rg_1) in a crude saponin preparations from Ginseng roots as well as some related compounds (sterols, bile acids and corticosteroids) has been reported[12–14].

The reagent gave stable red-purple colors λ_{max} in the region 515–545 nm.) with triterpenoid sapogenins, sterols (cholesterol α- & β-sitosterol) as well as bile acids and steroidal sapogenins which have an OH group at their C-3 position[14]. Cholanic acid (no OH groups) and corticosteroids did not give the typical red-purple color after treatment with the reagent. Therefore it has been concluded that the presence of hydroxyl group at C-3 is essential for the reaction[14], and that higher aliphatic alcohol and phenols also react with the reagent and give colors.

The objective of this study is to adapt the vanillin-sulfuric acid reagent for colorimetric assay of crude powdered myrrh and purified myrrh extract in different pharmaceutical formulations. Analytical quality criteria including linearity, precision, accuracy and recovery are discussed.

EXPERIMENTAL

Apparatus

Colorimetric determinations were performed using a Hewlett-Packard 8451 diode-array spectrophotometer with built-in functional keyboard and built-in printer/plotter and equipped with a 1-cm matched quartz cells.

Materials and Reagents

All experiments were performed with analytical grade chemicals and solvents.

1% w/v Vanillin Solution

This solution was freshly prepared by dissolving 1.0 g of vanillin in 100.0 mL of methanol.

25% v/v Sulfuric Acid Solution

This solution was freshly prepared.

The Oily Fraction

One Kilogram of crude powdered myrrh was extracted with petroleum ether (3×1.0 L) by percolation at room temperature, the combined extracts were evaporated under vacuum at 40°C to give oily fraction (A) as a pale yellow viscous liquid.

The Resin Extract

The defatted powder (obtained after extraction of crude powdered myrrh powder with petroleum ether) was extracted with methanol (3×1.0 L) by percolation at room temperature, the obtained yellowish brown residue was dried and powdered to give resin (B) as a yellow fine powder.

The Purified Myrrh Extract

Method "*a*" The oily fraction (A) and the resin extract (B) was mixed in calculated amounts to give the purified myrrh extract.

Method "*b*" One Kilogram of crude powdered myrrh was extracted with methyl alcohol (2×1.0L) by percolation at 40°C, the combined extracts were evaporated under vacuum at 50°C to give the purified myrrh extract. Purified myrrh extract used during this study as standard or in formulation of the capsules, suppositories and emulsion was that prepared by Method "a".

Volatile Oil

100 g of crude powdered myrrh was subjected to steam distillation according to B.P. 93 procedure[15].

Pharmaceutical Preparations

Herbal Mirazid soft gelatin capsules, suppositories and emulsion (Pharco pharmaceuticals, Alexandria, Egypt) labeled to contain 260 mg of purified myrrh extract per capsule, suppository or per 5.0 mL of emulsion were obtained.

Standard Solution and Calibration Graph

Stock standard solution of purified myrrh extract (5 mg/mL) was prepared in methanol and stored refrigerated at 4°C in brown glass flasks. Aliquots from stock standard solution (5 mg/mL) ranging from 400–2000 μL were transferred into 20- mL screw capped test tubes. To each flask, 5.0 mL of 1% w/v vanillin solution, 1.0 mL of 25% v/v sulfuric acid solution and 5.0 mL of methanol were added successively. The tubes were capped and heated in a water-bath at 60°C for 60 min, allowed to cool for 30 min, then the contents were transferred into 50- mL volumetric flasks and completed to volume with methanol. The absorbance was measured against a reagent blank at 518 nm using 1- cm cells.

Procedure for the Determination of Purified Myrrh Extract in Soft Gelatin Capsules, Suppositories, and Emulsion

With the point of a pair of sharp scissors, the end of twenty capsules were opened into a 100-mL beaker and mixed thoroughly. Twenty suppositories were accurately weighed, melted and mixed. A portion of mixed capsule content (or the melted suppository mass) or a 5.0 mL volume of the well- mixed oral emulsion, equivalent to about 260 mg purified myrrh extract was transferred to a 50-mL volumetric flask, dissolved in methanol and then completed to volume with methanol (and filtered in case of emulsion). Aliquots of this solution about (1000 μL) within the concentration range cited in Table 2 were treated as under calibration graph.

RESULTS AND DISCUSSION

The chromogenic reagent, ''vanillin-sulfuric acid'' has been found to produce a violet color with the purified myrrh extract. Although the vanillin- sulfuric acid reagent is not specific, as many compounds of different chemical structure react with this reagent[16], the violet colored reaction product formed with the purified myrrh extract exhibits a maximum absorbance at 518 nm. (Fig. 1), and has been found to be very stable for at least 6 hours. The chemistry of the violet colored product has not been elucidated; however, based on the previous findings[12–14], we could suggest that the reagent reacts with the terpenes of the volatile oil ''myrrhol'' such as elemol or guaiol (having OH-groups). This has been confirmed

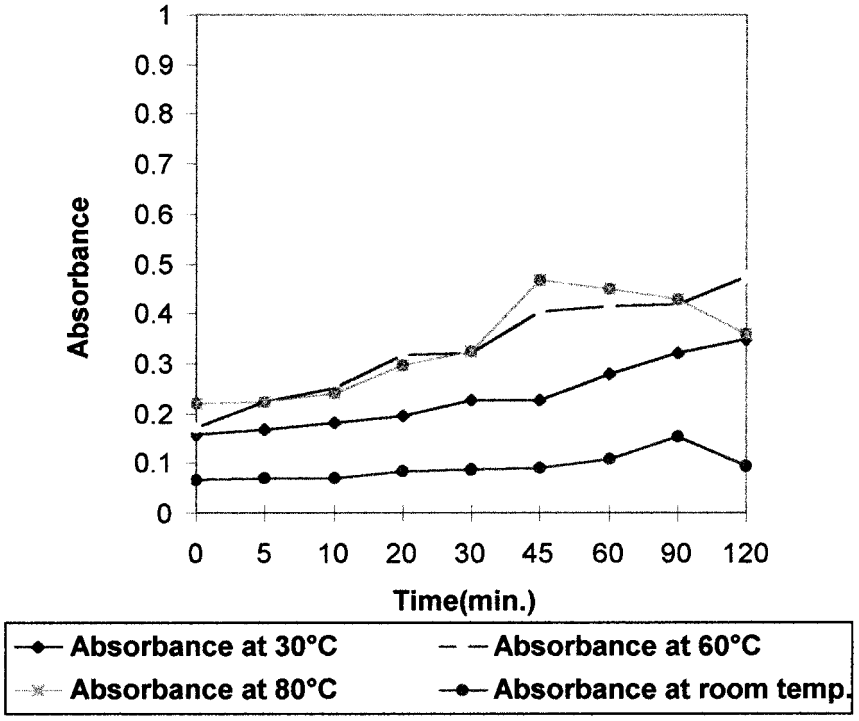

Figure 1. Absorbtion curve for the colored product of 100 μg/mL purified myrrh with vanillin-sulfuric acid.

by comparing the absorbance values of the reaction product of vanillin-sulfuric acid (or blue tetrazolium) reagent with equal quantities of each of the crude powdered myrrh, purified myrrh extract, oily fraction, volatile oil and resin (Table 1). It has been found, using the proposed vanillin-sulfuric acid method, that the highest absorbance value has been obtained with the oily fraction and volatile oil . The crude powdered myrrh gave lower absorbance value and no color has been produced with the resin extract. Using the blue tetrazolium method, the highest absorbance value has been obtained with the resin , while almost no reaction was observed with either the volatile oil or the oily fraction . These data (Table 1) proves that the proposed vanillin- sulfuric acid method is specific for assaying the oily fraction content of the crude powdered myrrh, while the blue tetrazolium method determines the resin content that is rich in steroids. Further study on the oily fraction using column chromatography, has proved that only

Table 1. Comparison of Absorbance Values of the Colored Reaction Products Obtained by the Proposed Vanillin-Sulfuric Acid Method and the Blue Tetrazolium Method with Each of Crude and Purified Myrrh and with the Different Solvent Extracts

Component Tested	Vanillin-Sulfuric Acid Method Absorbance* at 518 nm	Blue Tetrazolium Method Absorbance** at 525 nm
Volatile oil	0.500	0.005
Oily fraction (petroleum ether extract)	0.461	0.056
Purified myrrh extract	0.205	0.150
Crude powdered myrrh	0.101	0.324
Resin extract	0.004	0.932

* Absorbance values for the colored product of 40 μg/mL of each component.
**Absorbance values for the colored product of 5.7 μg/mL of each component.

certain subfractions gave a positive color with vanillin- sulfuric acid method. Also these subfractions were the only fractions that showed the molluscicidal activity against the snail *Biomphalaria alexandrina*, the intermediate host of *Schistosoma mansoni*.

Therefore it has been concluded that these subfractions of the oily fraction are the main bioactive fractions of the drug and that the proposed procedure is targeted towards determination of these bioactive components of the oily fraction in crude powdered myrrh or in its formulations.

Optimization of Reaction Variables

The optimum conditions for the development of the proposed colorimetric procedure were established by varying the reaction parameters one at a time, keeping the others fixed, and observing the effect produced on the absorbance reading at 518 nm.

The effect of vanillin reagent concentration on the color developed at 518 nm was attained by changing the volume of vanillin reagent 1% w/v solution over the range 1–7 mL .The use of 5.0 mL of 1% w/v solution was found to give constant and reproducible absorbance value (Fig. 2). Analogously, 1.0 mL of 25% v/v sulfuric acid solution was proven to be optimum for full color development (Fig. 3). The optimum heating temperature and heating time were found to be 60°C and 60 min, respectively (Fig. 4). Using the above described optimum experimental conditions the absorption

Table 2. Analytical Data of the Calibration Graph for the Determination of Purified Myrrh by the Proposed Method

		Linear Regression							
Wavelength λ (nm)	Concentration Range $\mu g\,mL^{-1}$	Intercept (a)	Slope (b)	Correlation Coefficient (r)	$S^*_{y/x}$	S_a**	S_b***	$C^{\dagger}_{L}$ $\mu g\,mL^{-1}$	$C^{\dagger\dagger}_{Q}$ $\mu g\,mL^{-1}$
518	40–200	0.0183	0.0044	0.9994	0.0115	0.0081	8.75×10^{-5}	1.104	3.679

*$S_{y/x}$ = Standard deviation of residuals.
**S_a = Standard deviation of intercept of regression line.
***S_b = Standard deviation of slope of regression line.
†$C_L = 3\ S_B/b$; C_L = Detection limit; S_B = Standard deviation of Blank; b = Slop of calibration graph.
††$C_Q = 10\ S_B/b$; C_Q = Quantification limit.

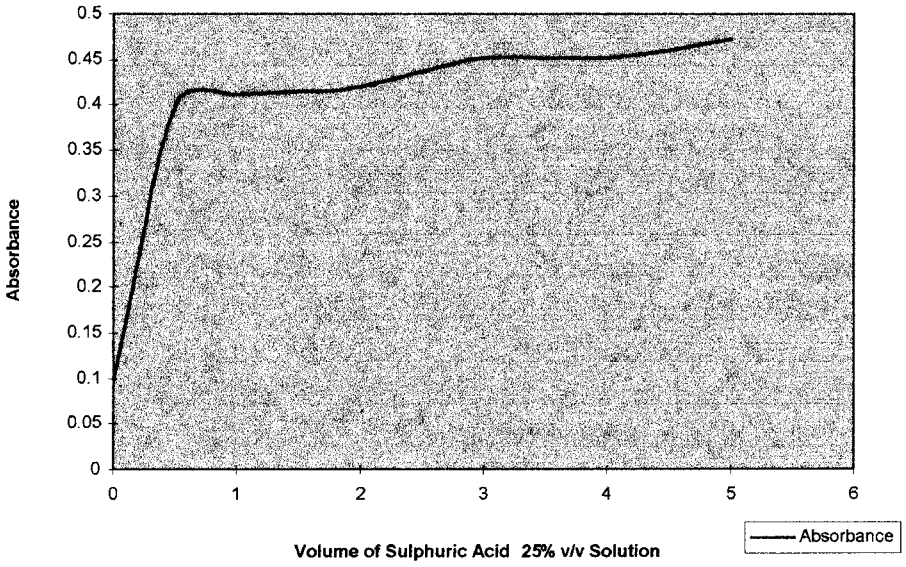

Figure 2. Effect of vanillin concentration on the color intensity developed from 100 μg/mL purified myrrh.

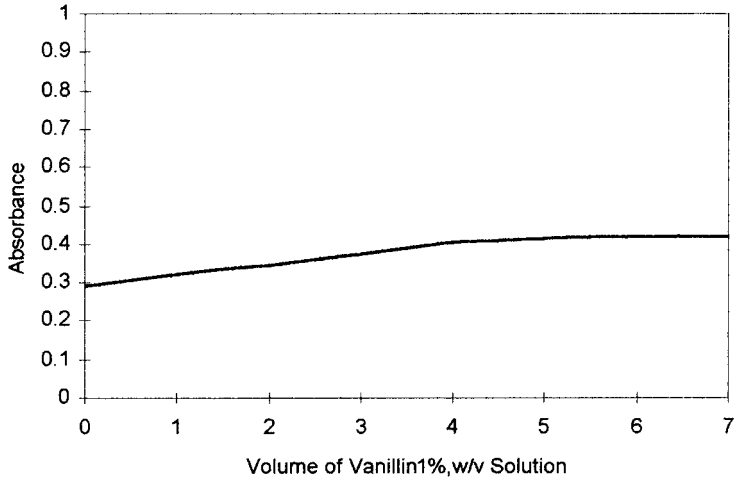

Figure 3. Effect of sulfuric acid concentration on the color intensity developed from 100 μg/mL purified myrrh.

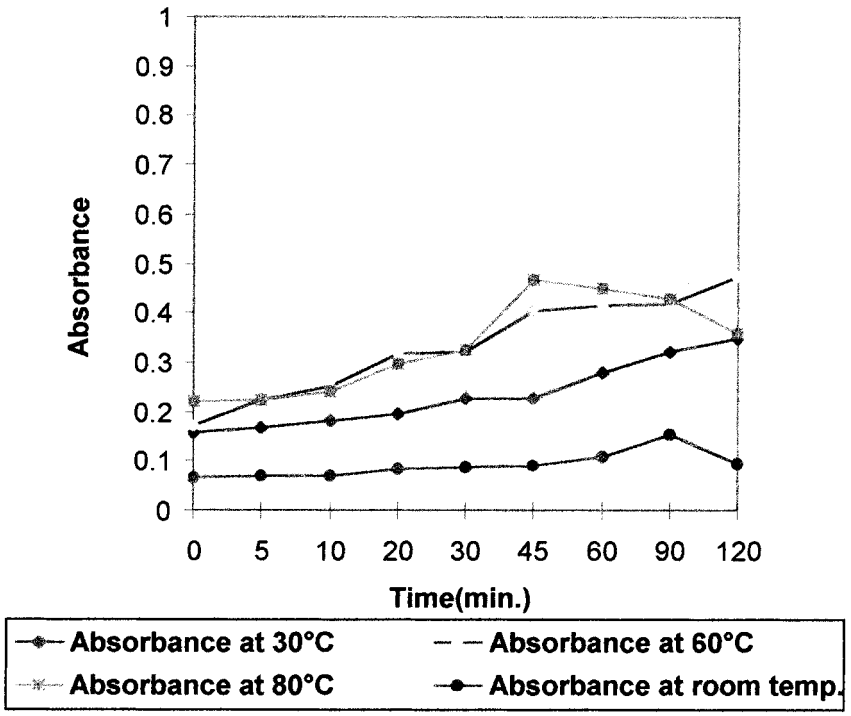

Figure 4. Effect of temperature & heating time on the color intensity developed from 100 μg/mL purified myrrh.

spectrum of the violet chromogenic species produced by the suggested procedure is shown to have maximum absorbance at 518 nm (Fig. 1).

Statistical Analysis of Results

The calibration graphs were constructed from at least five points over the concentration range 40–200 μg/mL of purified myrrh extract (Table 2). Regression analyses indicate linear relationship with negligible intercept. The results of the statistical analysis of the experimental data including regression equations calculated from calibration graphs, along with the standard deviation of the slope (S_b) and intercept (S_a) on the ordinate and the standard deviation of the residuals (Sy/x) are presented in Table 2. The high values of the correlation coefficients (r greater than 0.999) of the regression equation indicate good linearity and the conformity to Beer's law.

The small degree of scatter of the experimental data points around the line of regression is confirmed by the small value of the Sy/x. Because the intercepts on the y axis are close to zero, a single point calibration was justified.

Five replicate determination at 5 different concentration levels, were carried out to test the precision and accuracy of the proposed method. The relative standard deviation (RSD%) were found to be less than 2.15% indicating the excellent precision of the method and the relative standard errors (Er%) were found to be less than 3.55% indicating the high accuracy of the proposed method (Table 3).

The detection and quantification limits for the determination of purified myrrh extract by the proposed method were found to be 1.104 and 3.679 μg/mL, respectively (Table 2).

Extraction of Purified Myrrh Extract and Its Determination in Crude Powdered Myrrh

Quantitative extraction of the crude powdered myrrh by methanol (Method "a") has revealed that each 1000 mg of crude powdered myrrh yields 300 mg of purified myrrh extract. The latter has been prepared by another method called, Method "b", that is involving combination of the oily fraction and resin in calculated amounts. It has been proved, by the proposed colorimetric method, that each 300 mg of purified myrrh extract prepared by Method "a" is equivalent to 260 mg of that prepared by Method "b". Further more, applying the proposed colorimetric procedure to samples (n = 10) of crude powdered myrrh, has recovered 299 ± 0.26 mg of purified myrrh extract from each 1000 mg of crude powdered myrrh.

Table 3. Precision and Accuracy for the Determination of Purified Myrrh by the Proposed Method

Nominal Value ($\mu g\,mL^{-1}$)	Found ± SD ($\mu g\,mL^{-1}$)[a]	RSD (%)[b]	Er (%)[c]
40	41.42 ± 0.89	2.15	3.55
80	79.09 ± 1.16	1.49	−1.14
120	119.05 ± 1.24	1.04	−0.79
160	162.03 ± 2.34	1.44	1.27
200	198.91 ± 0.80	0.40	−0.55

[a] Mean ± standard deviation for five determinations.
[b] Percentage relative standard deviation.
[c] Percentage relative error.

Table 4. Determination of Purified Myrrh in Capsules, Suppositories, and in Emulsion

	(%Recovery)					
	Capsules		Suppositories		Emulsion	
	Vanillin-Sulfuric Acid	Blue Tetraz	Vanillin-Sulfuric Acid	Blue Tetraz	Vanillin-Sulfuric Acid	Blue Tetraz
	100.24	104.76	100.60	101.46	100.00	98.58
	101.21	103.57	100.36	100.29	99.76	101.98
	101.45	103.57	99.87	99.71	99.52	98.02
	101.70	102.98	99.83	101.46	100.24	98.02
	101.20	101.19	99.64	100.29	100.48	99.15
	101.45	104.17	100.60	98.54	100.24	103.68
	101.93	105.36	100.36	100.87	100.73	100.85
Mean ± SD[a]	101.31 ± 0.54	103.66 ± 1.35	100.18 ± 0.39	100.37 ± 1.04	100.14 ± 0.41	100.85 ± 2.19
RSD (%)[b]	0.53%	1.30%	0.39%	1.04%	0.41%	2.17%
Er (%)[c]	1.31%	3.66%	0.18%	0.37%	0.14%	0.85%
F[d]		6.26		7.20		28.24

[a] Average of 7 determination ; SD = Standard deviation.
[b] Percentage relative standard deviation.
[c] Percentage relative error.
[d] Theoretical value of F-test at P = 0.05 is 3.79.

These results by the colorimetric procedure are concordant with those obtained by the extraction procedure (Methods "a" and "b"). The above results proves that Mirazid can be formulated using either 260 mg of purified myrrh extract prepared by Method "a" or 300 mg of that obtained Method "b" per capsule, suppository or 5.0 mL emulsion.

Application of the Proposed Procedure to Pharmaceutical Analysis and Statistical Comparison of the Results

The proposed new analytical method was successfully applied to the colorimetric determination of purified myrrh extract in different pharmaceutical formulations (soft gelatin capsules, suppositories and emulsion). The results obtained were statistically compared by the variance ratio F-test[17], with those obtained by another colorimetric method[10] based on the reduction of blue tetrazolium salt. The latter has been applied for estimation of total steroidal content of *Commiphora mukul*. The variance ratio F-test values, calculated for $P = 0.05$ and $f_1 = f_2 = 7$, did exceed the theoretical value of $F = 3.79$ (a one tailed test) indicating that the vanillin-sulfuric acid proposed procedure is more precise than the blue tetrazolium method. All the results of the analysis of different dosage forms as well as those of the statistical comparison are represented in Table 4.

CONCLUSION

The above findings substantiate the usefulness of the proposed vanillin-sulfuric acid method for identification and determination of purified myrrh extract and more specifically the "oily fraction" either in crude drug or in dosage forms. The procedure is highly selective for identifying certain subfractions of the oily fraction that has been proved to be the major bioactive components of oily fraction which showed molluscicidal activity against the intermediate host of *Schistosoma mansoni*. Validation studies demonstrated that the procedure is simple accurate, linear, precise, reproducible and can be conveniently used for routine analytical work.

REFERENCES

1. Brieskorn, C.H.; Noble, P. Tetrahedron Letters **1980**, *21*, 1511.
2. Brieskorn, C.H.; Noble, P. Phytochemistry **1983**, *22* (1), 187.
3. Brieskorn, C.H.; Noble, P. Phytochemistry **1983**, *22* (5), 1207.

4. Massoud, A.; Salama, O.; Bennett, J.L. Proceeding of 9th International Congress of Parasitology, Makuhari Mess, Chiba, Japan, August 24–28, 1998.
5. Salama, O.; El. Sayed H. El. Ashry; Rashed, N.; Massoud, A.; El. Alqamy, H.E.M.; Saleh, A. Under Publication.
6. El. Gohary, Y.; Massoud, A.; Helmi, M.; Kassem, M.; Abdo, A.; Hanno, A.; Salama, O. Alexandria Medical Journal **1999**, *41* (1), 12.
7. Yu, X.; Ma, X.; Ding, X. Fenxi-Ceshi-Xuebao **1993**, *12* (2), 8.
8. Ma, X.; Yu, X.; Zheng, Z.; Mao, J. Yaowu-Fenxi-Zazhi **1992**, *12* (2), 83.
9. Ma, X.; Yu, X.; Zheng, Z.; Mao, J. Chromatographia **1991**, *32* (1–2), 40.
10. Rangari, V.D.; Donglikar, M.M. Indian Journal of Pharmaceutical Sciences **1994**, May–June, 110.
11. Cundiff, R.H. Analytical Chemistry **1964**, *36* (9), 1871.
12. Hiai, S.; Oura, H.; Homanaka, H.; Odaka, Y. Planta Medica **1975**, *28*, 131.
13. Hiai, S.; Oura, H.; Odaka, Y.; Nakajima, T. Planta Medica **1975**, *28*, 363.
14. Hiai, S.; Oura, H.; Nakajima, T. Planta Medica **1976**, *29*, 116.
15. British Pharmacopoeia 1993, HMSO, London **1993**, vol.II, A154.
16. Moffat, A.C.; Jackson, J.V.; Moss, M.S.; Widdop, B. *Clark's Isolation and Identification of Drugs*, 2nd ed.; The Pharmaceutical Press: London, 1986; 147.
17. Miller, J.C.; Miller, J.N. *Statistics for Analytical Chemistry*, 3rd ed.; Ellis Horwood: Chichester, 1993; 60.

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