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Validation of reagent-based paper test strips for paracetamol detection in herbal medicines: Response time and specificity

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Abstract:
Undeclared paracetamol is one of the active pharmaceutical ingredients most often found in Indonesian herbal medicines, yet official detection relies on laboratory chromatography. This study aimed to validate reagent-based paper test strips for paracetamol screening in herbal medicines by determining their response time and specificity. Whatman No. 1 paper was impregnated with 10% iron (III) chloride ($FeCl_3$) or Folin–Ciocalteu reagent by entrapment. Colour development was photographed at 5–60 min and quantified as red–green–blue (RGB) values with a color-picker application (n = 5). Specificity was assessed by comparing paracetamol standard (500 ppm), herbal sample (muscle-ache, gout and rheumatism products) and spiked solutions using color intensity, Euclidean color distance (ΔRGB) and Welch's t-test. A reading time of 30 min was optimal for both reagents. The unspiked herbal samples alone produced strong color responses and spiked and unspiked samples did not differ in five of six reagent–matrix combinations ($p > 0.05$). One rheumatism product gave a color intensity equivalent to the paracetamol standard with both reagents. Neither reagent therefore distinguished paracetamol from phenolic and reducing constituents of the herbal matrix. The strips may serve only as a preliminary, non-specific screening tool, and positive results require chromatographic confirmation.

Keywords:
paper-based analytical device; paracetamol; herbal medicine adulteration; specificity; RGB colorimetry

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INTRODUCTION

Herbal medicines (*jamu*) are widely used in Indonesia to maintain health and relieve common complaints, and the high demand has encouraged many manufacturers to market products with diverse therapeutic claims (BPOM, 2022). To protect consumers, BPOM Regulation No. 25 of 2023 prohibits the manufacture, import, and distribution of herbal medicines containing isolated or synthetic chemical substances, particularly active pharmaceutical ingredients (APIs) (BPOM, 2023). Nevertheless, the illegal addition of APIs to herbal and dietary products remains a global public health problem reported in Asia, Europe, and the Americas (Ernst, 2002; Calahan et al., 2016; Rocha et al., 2016).

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In Indonesia, post-market surveillance by BPOM, the national food and drug authority, has identified more than 40 adulterated herbal products every year, with the number increasing from 41 in 2022 to 50 in 2023 and 68 in 2025 (BPOM, 2025). Sildenafil citrate is the most common ingredient in products marketed for male stamina, whereas dexamethasone, phenylbutazone and paracetamol are typically found in products for muscle aches, rheumatism and gout. The risk is not trivial. Consumers do not know which drug they ingest, cannot control the dose, and may combine the product with over-the-counter analgesics. Paracetamol is safe at therapeutic doses, but repeated supratherapeutic exposure is a leading cause of drug-induced acute liver injury (Yoon et al., 2016). Hidden APIs also undermine the principles of safe medication use (right drug, right dose, and right patient) that health professionals apply in daily practice (Afandi et al., 2024), and they weaken the patient-safety culture of health services (Afandi et al., 2023).

The detection of APIs in herbal medicines currently relies on laboratory methods. The BPOM official procedure identifies prohibited APIs using thin-layer chromatography (TLC) followed by spectrophotometric densitometry (BPOM, 2018). These methods are reliable but require trained analysts, instruments, and time, which limits their use for routine field surveillance, community pharmacies, or district health offices. Paper-based analytical devices (PADs) offer an attractive alternative. Paper is inexpensive, lightweight and disposable, and its porous cellulose network wicks liquids and retains reagents without pumps or power (Martinez et al., 2010; Cate et al., 2015). Reagents immobilized on paper produce a color change when the analyte is applied, and a smartphone image can convert this color into numerical red–green–blue (RGB) values (Fan et al., 2021; Malik et al., 2024). PADs have already been used to screen falsified antibiotics and antituberculosis medicines in low-resource settings (Weaver et al., 2013).

Sentat et al. (2020) developed two prototype colorimetric paper test strips containing 10% iron (III) chloride ($FeCl_3$) or Folin–Ciocalteu reagent, and showed that both changed color in the presence of paracetamol in jamu. $FeCl_3$ forms coloured complexes with phenolic hydroxyl groups, whereas the Folin–Ciocalteu reagent is reduced by phenolic compounds to a blue molybdenum–tungsten species (Apak et al., 2016; Pérez et al., 2023). Because paracetamol carries a phenolic hydroxyl group, it reacts with both reagents. However, that method was not validated. Validation demonstrates that an analytical procedure is fit for its intended purpose, and specificity is a primary parameter: the ability to assess the analyte unequivocally in the presence of other components expected in the sample (ICH, 2023). Other paper-based methods have been validated for accuracy, detection and quantitation limits, precision, selectivity, and stability before application to real samples (Tsaftari et al., 2023). For herbal medicines, specificity is particularly critical, because plant matrices are rich in phenolics and other reducing compounds that react with the same reagents (Everette et al., 2010).

The chemical complexity of herbal preparations presents an additional analytical challenge because their composition varies with plant species, extraction procedures, formulations, and manufacturing processes. Natural constituents such as flavonoids, tannins, and other phenolic compounds may produce color reactions similar to those generated by paracetamol, potentially affecting the interpretation of colorimetric results. In addition, differences in the original color and physical characteristics of herbal formulations may influence visual assessment and smartphone-based RGB measurements. Consequently, a color response alone cannot confirm the presence of

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paracetamol unless the contribution of the herbal matrix has been adequately examined. Comparing standard solutions, unspiked herbal samples, and paracetamol-spiked samples provides a practical approach to distinguishing analyte-related responses from background matrix interference.

Two practical questions therefore remain unanswered: when should the color on these strips be read, and does the color reflect paracetamol rather than the herbal matrix? Without this evidence, a positive strip could wrongly implicate a legal product, and a negative strip could miss an adulterated one. Beyond analytical performance, establishing a reproducible color-reading time is essential for translating a paper-based test into a practical screening procedure. Color development may change over time as the reaction proceeds, making readings taken at different intervals difficult to compare. A defined response time can improve consistency between measurements and reduce subjective interpretation, particularly when the test is performed outside a fully equipped laboratory. When combined with specificity assessment, this information can establish the preliminary analytical suitability of the strips for screening herbal products. Nevertheless, such devices should be regarded as preliminary screening tools rather than definitive confirmatory methods, with suspicious results requiring further examination using validated laboratory techniques. This study aimed to validate reagent-based paper test strips (FeCl3 and Folin–Ciocalteu) for the detection of paracetamol in herbal medicines by determining (1) the optimal response time and (2) the specificity of the strips in three types of herbal medicines, products for muscle aches, gout and rheumatism, using standard, sample and spiked solutions.

METHODS

This laboratory-based experimental study evaluated two validation parameters of reagent-based paper test strips for paracetamol: response time and specificity. Specificity was defined, following ICH Q2(R2), as the ability of the strip to respond to paracetamol without interference from components of the herbal matrix (ICH, 2023). Each condition was tested in five independent replicates (n = 5). Iron (III) chloride hexahydrate (FeCl3·6H2O) and Folin–Ciocalteu reagent were purchased from Merck. Paracetamol reference standard (BPFI) was obtained from PPPOMN (Pusat Pengembangan Pengujian Obat dan Makanan Nasional). 96% ethanol was used as the solvent because paracetamol dissolves readily in it via hydrogen bonding with its amide and hydroxyl groups (Hakim & Saputri, 2020; Putra et al., 2023). Whatman No. 1 chromatography paper was used as the strip substrate. Three commercially available herbal medicines indicated for muscle aches (*pegal linu*), gout (*asam urat*), and rheumatism (*rematik*) served as test matrices.

A 10% (w/v) FeCl3 solution was prepared by dissolving 10 g of FeCl3·6H2O in distilled water in a 100 mL volumetric flask. The Folin–Ciocalteu reagent was prepared as described by Sentat et al. (2020). Reagents were immobilized by entrapment: Whatman No. 1 paper was cut into 2 × 3 cm strips, soaked in 5 mL of each reagent for 60 min, removed, and air-dried for 30 min at room temperature (Hermanto et al., 2020). The standard solution (500 ppm) was prepared by dissolving 12.5 mg of paracetamol standard in 96% ethanol in a 25 mL volumetric flask. Each sample solution was prepared in the same way from 12.5 mg of the herbal medicine. The spiked solution was prepared by dissolving 12.5 mg of paracetamol standard and 12.5 mg of the herbal

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medicine together in 96% ethanol in a 25 mL volumetric flask. The spiked solution served as a positive matrix control: if the strip were specific, its color would differ clearly from that of the unspiked sample and approach that of the standard.

Two drops of the paracetamol standard solution were applied to each FeCl3 and Folin–Ciocalteu strip. Images were captured at 5, 15, 30, 45 and 60 min after application. The optimal response time was defined as the reading time at which color development was complete and inter-replicate variability was lowest, before color drift occurred. An aliquot of 0.05 mL of the standard, sample, or spiked solution was applied to separate strips for each reagent and each type of herbal medicine. Color was read at the optimal response time determined in the previous step (30 min).

All images were captured under the same lighting conditions to minimize environmental variation. The reaction zone of each strip was analyzed using a color picker application that returned red (R), green (G), and blue (B) values on a 0–255 scale. Lower values indicate a darker, more intense color.

RGB values are reported as mean ± standard deviation (SD) of five replicates. Overall color intensity was calculated as $I = (R + G + B)/3$, and the difference between two colors as the Euclidean distance $\Delta RGB = \sqrt{[(R_1 - R_2)^2 + (G_1 - G_2)^2 + (B_1 - B_2)^2]}$ (Fan et al., 2021). Repeatability at each reading time was expressed as the coefficient of variation (CV%) of intensity. Changes across reading times were tested with the Friedman test. In the specificity test, color intensities of the standard versus sample and of the sample versus spiked solutions were compared with Welch's t-test. Statistical significance was set at $p < 0.05$. Analyses were performed in Python (SciPy).

RESULTS

Color responses were quantified as R, G, and B values, where lower values indicate a darker color. Raw replicate data are shown in Tables 1 and 3, and summary statistics in Tables 2 and 4. Table 1 presents the raw RGB values of the paracetamol standard on both strips from 5 to 60 min.

For $FeCl_3$, the mean color changed most between 15 and 30 min ($\Delta RGB = 15.4$) and only slightly thereafter ($\Delta RGB = 6.2–9.2$). The CV of color intensity fell from 21.0–22.8% at 5–15 min to 13.3% at 30 min (Table 2). The R ($p = 0.004$) and G ($p = 0.037$) channels changed significantly over time, whereas the B channel and overall intensity did not ($p \geq 0.578$). For Folin–Ciocalteu, color intensity was lowest, that is, the color was most intense, at 30 min (81.1 ± 10.4). After 30 min, the color drifted markedly ($\Delta RGB = 30.6$ between 30 and 45 min and 27.6 between 45 and 60 min), and variability increased to 14.3–16.2%; no channel changed significantly over time ($p \geq 0.062$). Based on these findings, 30 min was selected as the optimal response time for both reagents.

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Table 1. Response time test results: raw RGB values of the paracetamol standard (n = 5)

Reagent	Replication	Response Time (minutes)	Red, Green, and Blue (RGB) color values		
			R	G	B
Iron (III) chloride	1	5	164	171	122
		15	171	175	125
		30	168	170	79
		45	176	176	90
		60	181	178	75
	2	5	153	156	80
		15	141	145	67
		30	167	167	99
		45	169	166	79
		60	174	168	58
	3	5	129	132	44
		15	120	122	9
		30	141	142	52
		45	128	122	40
		60	148	140	49
	4	5	163	167	107
		15	167	170	85
		30	175	175	95
		45	181	180	103
		60	183	179	97
	5	5	139	141	0
		15	143	145	54
		30	143	143	55
		45	152	147	40
		60	157	149	59
Folin ciocalteu	1	5	102	118	39
		15	95	111	30
		30	98	110	50
		45	92	109	45
		60	105	120	62
	2	5	122	137	62
		15	105	125	40
		30	72	92	25
		45	127	145	89
		60	77	96	47
	3	5	100	117	43
		15	97	113	50
		30	99	115	52
		45	105	120	63
		60	72	93	35
	4	5	97	117	35
		15	88	107	28
		30	95	110	52
		45	109	124	72
		60	88	106	50
	5	5	91	111	32
		15	96	115	31
		30	92	108	47
		45	102	118	60
		60	104	122	65

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Table 2. Summary of color development over time (mean ± SD, n = 5)

Reagent	Time (min)	R	G	B	Intensity (I)	CV (%)	ΔRGB*
$FeCl_3$	5	149.6 ± 15.3	153.4 ± 16.7	70.6 ± 49.4	124.5 ± 26.2	21.0	–
	15	148.4 ± 20.9	151.4 ± 21.5	68.0 ± 42.5	122.6 ± 27.9	22.8	3.5
	30	158.8 ± 15.7	159.4 ± 15.7	76.0 ± 21.9	131.4 ± 17.4	13.3	15.4
	45	161.2 ± 21.6	158.2 ± 23.9	70.4 ± 29.0	129.9 ± 24.4	18.7	6.2
	60	168.6 ± 15.4	162.8 ± 17.5	67.6 ± 18.9	133.0 ± 16.5	12.4	9.2
	p-value	0.004	0.037	0.750	0.578		
Folin–Ciocalteu	5	102.4 ± 11.7	120.0 ± 9.9	42.2 ± 11.8	88.2 ± 11.1	12.6	–
	15	96.2 ± 6.1	114.2 ± 6.7	35.8 ± 9.2	82.1 ± 6.3	7.6	10.6
	30	91.2 ± 11.1	107.0 ± 8.8	45.2 ± 11.5	81.1 ± 10.4	12.8	12.9
	45	107.0 ± 12.8	123.2 ± 13.4	65.8 ± 16.2	98.7 ± 14.1	14.3	30.6
	60	89.2 ± 15.1	107.4 ± 13.3	51.8 ± 12.1	82.8 ± 13.4	16.2	27.6
	p-value	0.395	0.275	0.062	0.251		

Values are mean ± SD (n = 5). I = (R + G + B)/3; lower values indicate a darker color. CV = coefficient of variation of I.
*ΔRGB = Euclidean distance between the mean color at that time and the mean color at the previous reading time.

Table 3. Specificity test results: raw RGB values (R, G, B) of standard, sample and spiked solutions

Reagent	The Types of Herbal medicine	Replication	Red, Green, and Blue (RGB) color values		
			Standard	Sample	Spike
Iron (III) chloride	Muscle aches	1	180,179,100	76,65,18	109,98,48
		2	184,186,139	116,103,26	108,97,47
		3	176,168,0	117,102,37	108,97,49
		4	183,180,46	107,95,22	103,89,35
		5	183,178,60	123,108,50	132,121,18
	Gout	1	143,144,64	115,91,41	124,104,63
		2	146,148,65	119,95,52	117,97,57
		3	156,157,88	123,100,61	109,90,53
		4	148,149,31	96,72,34	99,77,25
		5	154,158,67	113,90,41	118,98,41
	Rheumatism	1	149,145,53	152,131,25	135,121,59
		2	150,143,36	157,136,21	143,128,19
		3	174,175,110	174,152,78	154,139,96
		4	170,162,59	168,148,56	154,143,63
		5	146,133,9	151,127,62	127,113,47
Folin ciocalteu	Muscle aches	1	126,146,80	102,98,5	107,108,39
		2	111,132,78	83,74,0	93,88,19
		3	123,141,85	83,80,0	81,84,12
		4	112,129,64	83,76,0	89,89,8
		5	106,124,68	73,69,0	82,82,2
	Gout	1	71,88,39	74,77,31	68,70,29
		2	92,109,59	78,84,36	82,86,30
		3	84,100,41	69,72,25	63,68,24
		4	90,108,37	74,75,22	52,46,24
		5	77,91,43	76,80,26	56,55,23
	Rheumatism	1	78,95,27	129,116,0	106,95,0
		2	91,105,36	114,100,0	106,96,0
		3	84,101,37	123,114,0	106,96,0
		4	100,119,46	135,124,0	93,88,1
		5	106,120,50	123,109,0	120,110,0

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The specificity test examined whether the strips responded to paracetamol without interference from other constituents of the herbal medicines. Raw RGB values are presented in Table 3. With $FeCl_3$, the unspiked muscle-ache and gout samples were markedly darker than the paracetamol standard (both $p < 0.001$), while their spiked solutions did not differ from the samples ($p = 0.429$ and $p = 0.782$; $\Delta RGB = 11.3$ and 4.1). The rheumatism sample had an intensity similar to that of the standard (115.9 ± 13.5 vs 120.9 ± 21.8; $p = 0.672$), and spiking did not cause a significant change ($p = 0.493$). With Folin–Ciocalteu, the muscle-ache and gout samples were again darker than the standard ($p < 0.001$ and $p = 0.008$), with no sample–spike difference ($p = 0.136$ and $p = 0.144$). The rheumatism sample showed the same intensity as the standard (79.1 ± 5.5 vs 79.7 ± 10.4; $p = 0.923$) but a different hue ($\Delta RGB = 51.4$), and spiking produced a darker color ($p = 0.013$). Overall, spiked and unspiked samples were indistinguishable in five of six reagent–matrix combinations (Table 4).

Table 4. Statistical comparison of color responses in the specificity test

Reagent	Herbal medicine	Standard (I)	Sample (I)	Spiked (I)	ΔRGB standard –sample	ΔRGB sample –spiked	p standard vs sample	p sample vs spiked
FeCl3	Muscle aches	142.8 ± 20.4	77.7 ± 15.4	83.9 ± 5.3	117.7	11.3	<0.001	0.429
	Gout	121.2 ± 9.3	82.9 ± 10.2	84.8 ± 11.2	73.5	4.1	<0.001	0.782
	Rheumatism	120.9 ± 21.8	115.9 ± 13.5	109.4 ± 14.9	14.0	22.1	0.672	0.493
Folin–Ciocalteu	Muscle aches	108.3 ± 8.2	55.1 ± 7.9	65.5 ± 11.5	97.2	19.3	<0.001	0.136
	Gout	75.3 ± 7.9	59.9 ± 4.1	51.7 ± 9.9	28.1	16.2	0.008	0.144
	Rheumatism	79.7 ± 10.4	79.1 ± 5.5	67.8 ± 5.7	51.4	24.3	0.923	0.013

Values are mean ± SD (n = 5). I = (R + G + B)/3. ΔRGB = Euclidean distance between mean colors. p values from Welch's t-test on color intensity; $p > 0.05$ between sample and spiked solutions indicates that the strip did not discriminate added paracetamol from the matrix.

Comparison of the color responses across the three herbal medicine types also revealed different patterns of matrix interference. The muscle-ache and gout formulations consistently produced darker responses than the paracetamol standard with both reagents, indicating substantial background color contributions from the herbal matrices. In contrast, the rheumatism formulation produced responses comparable in intensity to the standard, particularly with FeCl3 and Folin–Ciocalteu, although differences in color composition were also observed. The addition of paracetamol did not consistently produce a distinguishable response across the tested formulations. These findings demonstrate that the colorimetric responses were strongly dependent on the herbal matrix and that neither reagent consistently distinguished unspiked from paracetamol-spiked samples.

DISCUSSION

This study evaluated two validation parameters of FeCl3- and Folin–Ciocalteu-based paper test strips for paracetamol. Both strips produced a measurable color with the paracetamol standard, and 30 min emerged as the most suitable reading time. However, the specificity test showed that the herbal matrices themselves produced strong color responses and that adding paracetamol did

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not change the color in a consistent or statistically meaningful way. In simple terms, the strips can show that a reactive substance is present, but not that this substance is paracetamol.

Response time matters because color tests are read by eye or camera at a single moment. For FeCl3, the color changed most between 15 and 30 min and then shifted only slightly, while inter-replicate variability fell from more than 20% at 5–15 min to 13.3% at 30 min. This pattern suggests that complex formation between Fe3+ and the phenolic hydroxyl group of paracetamols, together with evaporation of the ethanol on the paper, was largely complete by 30 min. The gradual increase in R and G values afterward indicates slight fading of the colored complex, consistent with the limited stability of ferric–phenolate complexes exposed to air and light (Apak et al., 2016). Readings taken too early capture an incomplete reaction, whereas very late readings may capture fading.

The Folin–Ciocalteu strip behaved differently. Its color was most intense at 30 min, but large shifts occurred between 30 and 60 min and variability increased after 15 min. The Folin–Ciocalteu reaction is an electron-transfer reaction in which phosphomolybdic–phosphotungstic acids are reduced to blue species; in solution it needs an alkaline medium, usually sodium carbonate, to proceed fully (Everette et al., 2010; Pérez et al., 2023). On a dry paper strip without added base, the reaction is probably slower and less controlled, and the reduced complex may partly re-oxidize, which would explain the drift. For both reagents, 30 min therefore represents a practical balance between complete color development and color stability.

Specificity is the central question for any screening test intended for regulatory surveillance. A specific strip would give a light color with an unadulterated sample and a clearly darker color after spiking. This was not observed. For the muscle-ache and gout products, the unspiked samples were already much darker than the paracetamol standard (ΔRGB = 28–118), and the spiked solutions were almost indistinguishable from the samples (ΔRGB = 4–19). The matrix effectively saturated the signal, leaving no room for paracetamol to make a visible difference. The rheumatism product revealed the opposite problem. With both reagents, the unspiked sample had practically the same intensity as the standard ($p = 0.672$ and $p = 0.923$). A field officer reading these strips would classify this product as positive for paracetamol. Whether the product truly contained paracetamol cannot be decided from color alone and requires confirmation by TLC–densitometry or high-performance liquid chromatography (BPOM, 2018). The single significant sample–spike difference (Folin–Ciocalteu, rheumatism; $p = 0.013$) does not establish specificity, because the unspiked sample already mimicked the standard.

The lack of specificity is explained by the chemistry of both reagents. The Folin–Ciocalteu reagent does not recognize a particular molecule; it measures total reducing capacity. Everette et al. (2010) showed that it reacts not only with phenolics but also with ascorbic acid, certain amino acids, proteins, aromatic amines, reducing sugars and other compounds. Sánchez-Rangel et al. (2013) and Pérez et al. (2023) likewise reported that the assay overestimates phenolic content in plant matrices because of such interferences, which is why it is classified as an electron-transfer capacity assay rather than a compound-specific test (Apak et al., 2016). FeCl3 is also a group reagent: Fe3+ forms colored complexes with almost any phenolic hydroxyl group, including those of flavonoids, tannins and phenolic acids. Herbal products for musculoskeletal complaints are commonly made from rhizomes, barks and leaves that are rich in these compounds. Paracetamol,

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a simple para-substituted phenol, therefore, competes with a large pool of matrix phenolics for the same reagent, and its contribution disappears within the overall signal.

These findings refine those of Sentat et al. (2020), who reported that the same reagents changed color with paracetamol in jamu but did not test unspiked matrices against spiked controls. Without these controls, a color change can easily be attributed to the drug when it actually comes from the plant material. The present results show that the matrix control is not optional; it is the decisive experiment. This agrees with the general principle that a paper-based color test is only as selective as the chemistry immobilized on it (Cate et al., 2015; Malik et al., 2024). Pharmaceutical PADs that work well in the field have achieved selectivity by combining several reagents on one card to create a color "fingerprint" (Weaver et al., 2013), by separating the analyte from the matrix before detection, or by coupling paper with electrochemical detection (Primpray et al., 2019; Costa-Rama & Fernández-Abedul, 2021). Fully validated paper methods, such as the fluorometric quinine assay by Tsaftari et al. (2023), also report selectivity for matrix components before being applied to real samples.

An important implication of these findings is that analytical sensitivity and analytical specificity should be considered separately when evaluating a colorimetric screening device. The ability of both strips to produce measurable responses to paracetamol standards demonstrates that the reagents are chemically responsive to the target compound under the tested conditions. However, this response does not establish that the strips can distinguish paracetamol from other substances in a complex herbal formulation. In practical terms, a strong color response may indicate the presence of phenolic or reducing compounds rather than the target pharmaceutical ingredient. Therefore, the analytical performance of a screening device should not be judged solely by the intensity of its color response or its ability to detect a standard solution. Its ability to discriminate the target analyte from relevant matrix components is equally important before it can be considered suitable for routine screening.

The color readout itself deserves attention. RGB values obtained from images are sensitive to illumination, camera white balance, and the area selected on the strip (Fan et al., 2021). Constant lighting and replicate strips reduced these effects, but the SD values in Tables 2 and 4 show that variation between strips remained considerable. Standardizing the image background, including a reference color card and converting RGB values to a perceptually uniform space such as CIELAB, would improve robustness (Fan et al., 2021). Paper properties also matter. Whatman No. 1 paper has a moderate pore size and a uniform surface that distributes reagents evenly (Buking et al., 2019), and entrapment keeps the reagent within the fiber network with minimal loss during analysis (Hermanto et al., 2020). These features support a visible color response, but they cannot confer the selectivity the reagent itself lacks.

From a public health and nursing perspective, the message is clear. Adulterated herbal products expose consumers to hidden drugs at unknown doses (Calahan et al., 2016; Rocha et al., 2016), and repeated hidden intake of paracetamol may contribute to liver injury, especially when combined with regular analgesic use (Yoon et al., 2016). Nurses, pharmacists, and community health workers who ask patients about herbal use should not rely on these strips to determine whether a product is safe or unsafe. A non-specific test can produce false positives that harm legitimate producers, and its results can create misplaced confidence. Medication safety depends

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on knowing the right drug and the right dose (Afandi et al., 2024), and a strong safety culture depends on reliable information (Afandi et al., 2023). The strips may still have a modest role as an inexpensive first step that flags products for laboratory confirmation.

For practical implementation, the development of paper-based screening devices should prioritize analytical discrimination, reproducibility, and ease of interpretation. One possible strategy is to incorporate a preliminary separation or selective extraction step that reduces interference from naturally occurring phenolic compounds before the detection reaction. Another approach is to use multiple reaction zones with different chemical principles, allowing the resulting color patterns to be interpreted collectively rather than relying on a single color response. Smartphone-assisted analysis could further support standardized interpretation by incorporating reference colors, controlled illumination, and predefined decision thresholds. Nevertheless, these improvements would require systematic analytical evaluation using representative herbal products and chromatographic confirmation to establish their reliability. Such development is particularly relevant for resource-limited surveillance settings, where an affordable screening method could support the identification of products requiring further laboratory investigation.

This study has several limitations. A small number of commercial products were tested, a single paracetamol concentration was used, and other validation parameters, limit of detection, precision, accuracy, and robustness, were not assessed. In addition, the paracetamol content of the samples was not confirmed by a chromatographic reference method. Future studies should include products confirmed to be free of and to contain paracetamol, test several spiking levels to estimate detection limits, add a clean-up or separation step on the paper to remove phenolic interferences, and explore more selective detection, such as multi-reagent color fingerprints, electrochemical paper sensors or paper-based mass spectrometry, which can detect paracetamol within minutes (Pradela-Filho et al., 2023; Zhou et al., 2024).

CONCLUSION

Reagent-based paper test strips prepared with $FeCl_3$ or Folin–Ciocalteu reagent produced a readable color with paracetamol, and 30 min was the optimal reading time for both. However, both strips failed the specificity test: the herbal matrices produced colors similar to or more intense than paracetamol, and spiking did not consistently alter the color. Therefore, these strips cannot specifically detect paracetamol in herbal medicines and should be used only as a preliminary, non-specific screening tool, with positive or doubtful results confirmed by chromatographic methods.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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