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Innovation of Colorimetric Test Strips to Detect Paracetamol and Dexamethasone in Jamu Preparations

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ABSTRACT

Background: The addition of chemical drugs to traditional herbal medicines (jamu) remains a public health concern because consumers may unknowingly be exposed to substances that can cause harmful effects. Paracetamol and dexamethasone are among the chemical drugs commonly misused in jamu products for pain, rheumatism, and gout. **Objective:** This study aimed to develop colorimetric test strips for the rapid qualitative detection of paracetamol and dexamethasone in jamu preparations. **Methods:** Laboratory experimental research was conducted using Whatman No. 4 filter paper as the test-strip medium. The paper was immobilized with 10% iron (III) chloride ($FeCl_3$) for paracetamol detection and a reagent mixture of anhydrous acetic acid, concentrated sulfuric acid, and iodine for dexamethasone detection. Five jamu samples for aches, pain, and gout obtained from Pasar Kliwon, Temanggung, Central Java, were tested. Standard paracetamol and dexamethasone solutions served as positive controls, while distilled water was used as a negative control. **Results:** Paracetamol produced a yellowish-green color response with $FeCl_3$, whereas dexamethasone produced a dark brown to reddish-brown color response with the reagent mixture. Four of the five tested jamu samples showed positive results for at least one chemical drug, while one sample was negative for both analytes. **Conclusion:** The developed colorimetric test strips have potential as a practical, rapid, and economical preliminary screening method for detecting paracetamol and dexamethasone in jamu. Further validation is required to assess selectivity, accuracy, precision, detection limit, and stability.

Keywords : medicinal chemicals, paracetamol, dexamethasone, test strip, colorimetry, traditional herbal medicine.

INTRODUCTION

Indonesia is a country with an abundance of medicinal plants, making herbal medicine a cultural heritage that has been used for generations by the community to maintain health and treat various diseases. Jamu is a traditional medicinal product whose ingredients are derived from plants, animals, minerals, galenic preparations, or mixtures of these ingredients, which have traditionally been used for treatment based on experience. According to the Ministry of Health of the Republic of Indonesia (2019), the development of traditional medicine in Indonesia shows a continuing upward trend, both in terms of the number of products in circulation and the level of public consumption, reflecting high trust in herbal-based medicine. Jamu is widely known by the public because it is considered safer, cheaper, and easier to obtain than conventional medicines from pharmacies. The population of Indonesians who are traditionally accustomed to using herbal medicine continues to increase along with the increasing awareness of a healthy, nature-based lifestyle. (Perwito et al., 2020) . The most widely consumed types of herbal medicine include herbal medicine for aches and pains, herbal medicine for gout, herbal medicine for rheumatism, herbal medicine for weight gain, and herbal medicine for slimming, each of which is claimed to have its own benefits by both producers and consumers (Wibowo, 2019) Internationally, the growing use of traditional and complementary medicine has reinforced the need for product regulation, quality assurance, and safety monitoring (World Health Organization, 2019).

BKO are ingredients that fall into the category of prescription drugs or prescription drugs that are not permitted to be added to traditional medicine preparations. BKO is intentionally added to herbal medicines by irresponsible manufacturers with the aim of providing a fast and strong pharmacological effect so that consumers perceive the herbal medicine as highly effective, when in fact, these effects do not come from the natural ingredients but from hidden chemical drug content. (PUTI PRIYANA et al., 2023) . This practice is highly detrimental to public health because consumers are aware that they are consuming prescription drugs without medical supervision, thus posing a high risk of side effects, drug interactions, and health complications. The Indonesian Food and Drug Monitoring Agency (BPOM RI) consistently monitors the distribution of traditional medicines containing BKO, and findings indicate that this problem remains unresolved. Based on BPOM findings, from September 2022 to October 2023, more than 50 traditional medicines and health supplements containing BKO, totaling over one million pieces and having an economic value of over IDR 39 billion, were found in various regions across Indonesia, including Central Java, East Java, Riau, North Sumatra, and South Sulawesi (RI Ministry of Health. (2019), 2022) .

Various types of OTCs have been found in traditional herbal preparations sold on the Indonesian market. Based on research and monitoring conducted in various regions, the most frequently found OTCs include paracetamol, dexamethasone, prednisone, piroxicam, meloxicam, diclofenac sodium, mefenamic acid, and sibutramine hydrochloride (Caroline Theodora et al., 2025; Fanani & Yunitasari, 2025; Trisiani et al., 2026) . Paracetamol, or acetaminophen, is one of the most widely used analgesic-antipyretic drugs worldwide due to its effectiveness in relieving mild to moderate pain and reducing fever. Chemically, paracetamol (4-acetamidophenol) is synthesized through the acetylation process of p-aminophenol using acetic anhydride as the acetylating agent (Sandrina & Ahmad Fauzan, 2023) . This drug works by inhibiting prostaglandin synthesis in the central nervous system and modulating the endocannabinoid system, thus providing analgesic and antipyretic effects without the significant anti-inflammatory effects of NSAIDs. Paracetamol is widely available without a prescription in various dosage forms, including tablets, capsules, syrups, and suppositories, and is widely used in various population groups, including pregnant women (Zeid et al., 2024) . Although considered safe at recommended therapeutic doses (500-1000 mg per dose, maximum 4 grams per day in adults), uncontrolled and excessive use of paracetamol can cause severe and potentially fatal liver toxicity. The hepatotoxic effect of paracetamol occurs due to excessive metabolism to form the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which is toxic to liver cells (Ghosh et al., 2021) . The risk of hepatotoxicity is increased in individuals who consume alcohol or have pre-existing liver disease.

This condition is highly prevalent because herbal remedies for aches and pains are often consumed daily by people experiencing chronic pain, who unknowingly accumulate paracetamol doses exceeding safe limits. Furthermore, long-term, unsupervised paracetamol use is also associated with an increased risk of kidney and cardiovascular disorders, and in vulnerable groups such as pregnant women, it can affect fetal development (Zeid et al., 2024) .

Dexamethasone is a synthetic corticosteroid belonging to the glucocorticoid group that has very strong anti-inflammatory and immunosuppressive potential, approximately 25 times more potent than endogenous cortisol. In the medical world, dexamethasone is widely used for various clinical indications such as the management of severe inflammatory conditions, autoimmune diseases, cerebral edema, severe allergic conditions, and as an adjuvant in post-operative pain management. Several recent clinical studies have demonstrated the effectiveness of dexamethasone in reducing post-operative opioid consumption in various surgical procedures including total knee arthroplasty, hip arthroplasty, periacetabular osteotomy, and pediatric tonsillectomy (Gasbjerg et al., 2024) . This powerful anti-inflammatory effect of dexamethasone is why this BKO is often used illegally in herbal preparations to provide rapid and dramatic pain relief and anti-inflammatory effects. When someone consumes herbal medicine containing hidden dexamethasone, they will feel lighter, pain reduced, and general condition improved quickly, which then gives the impression that the herbal medicine is very effective, when in fact the benefits come entirely from the dexamethasone, not from the herbal ingredients (Caroline Theodora et al., 2025) .

Long-term, unsupervised use of dexamethasone has a very serious and potentially life-threatening side effect profile. Dexamethasone is immunosuppressive, so long-term use causes a decrease in the immune system, making individuals susceptible to opportunistic infections. Other systemic side effects include Cushing's syndrome (moon face, buffalo hump, abnormal fat distribution), hyperglycemia and worsening of diabetes mellitus, hypertension, osteoporosis, growth retardation in children, posterior subcapsular cataracts, adrenal dysfunction (HPA axis suppression), and steroid psychosis (Maulida, 2022; Permadi, 2018) .

MATERIALS AND METHOD

Materials

Research Tools and Materials

The tools used were measuring cylinders (5 ml, 10 ml, and 100 ml), glass funnels (Herma), 250 ml Erlenmeyer flasks (Herma), 250 ml beakers (Herma), volumetric pipettes, volumetric flasks (10 ml, 50 ml, and 100 ml) (Iwaki), dropper pipettes, 250 ml porcelain cups, test tubes (Herma), test tube racks, analytical balances (Ohaus), blenders, scales (Krischef), cellulose paper, medicinal chemicals, and the materials used were FeCl3, H2SO4 reagent + anhydrous acetic acid + iodine, and medicinal chemicals.

Sample Collection

In this research, five herbal medicines for aches and pains and gout were collected from Kliwon Market in Temanggung , Central Java.

Sample Solution Preparation

100 ml of distilled water was added to the samples and stirred. The herbal samples were filtered and collected in beakers.

Reagent Strip Test

Liebermann Burchard

The dexamethasone screening reagent was prepared from concentrated sulfuric acid, acetic acid, and iodine solution. The reagent mixture was applied as the dexamethasone test-strip reagent.

Iron (III) chloride 10%

Weigh 10 g of iron (III) chloride, dissolve in distilled water, and dilute to 100 ml.

Test Strip Preparation

Whatman No. 4 filter paper was cut into 1 x 1 cm pieces. Each paper piece was immersed in the corresponding reagent solution and dried until completely dry before use.

Test Strip Reagent Suitability

The reagent suitability test is conducted by comparing the color changes that occur from the reaction between the reference solutions (Nurhasnawati et al., 2020) of Paracetamol and Dexamethasone with several reagents. A negative control test is performed using distilled water. If a color change is found, the reagent is considered suitable for test strip preparation

RESULTS

1. Test Strip Development and Reagent Suitability

Colorimetric test strips were prepared by immobilizing the designated reagents on Whatman No. 4 filter paper. The suitability assessment showed a visible color change for both reagent systems after contact with their respective reference solutions. The 10% FeCl3 strip changed from yellow to orange, while the dexamethasone reagent strip changed from light brown to dark brown (Table 1). These observations indicate that both immobilized reagent systems remained visually responsive under the testing conditions.

Table 1. Test strip reagent suitability results

Test strip	Initial color	Final color	Response
Dexamethasone reagent strip	Light brown	Dark brown	Color change observed (+)
10% FeCl3 strip	Yellow	Orange	Color change observed (+)

Note: (+) indicates that a visible color change was observed. The recorded colors follow the original experimental table.

2. Colorimetric Response of Reference Solutions and Controls

The direct qualitative reaction of the paracetamol reference solution with 10% FeCl3 produced a yellowish-green response, as shown in Figure 1. In contrast, the immobilized strip was recorded as changing from yellow to orange in the suitability test. For dexamethasone, the direct reaction of the acetic acid, concentrated sulfuric acid, and iodine mixture was described as producing a reddish-brown to purple response, whereas the strip observation was recorded as a light-brown to dark-brown change. The negative controls did not meet the study criteria for a positive test result, while the positive controls produced the color responses classified as positive in Table 2.

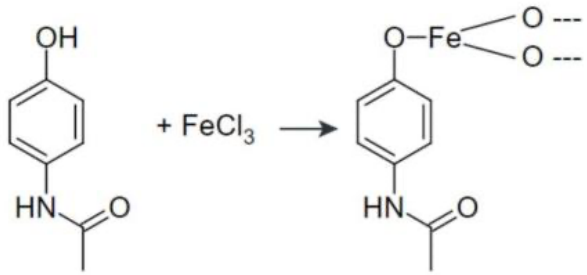

Figure 1. Illustrative reaction of paracetamol with FeCl3.

3. Screening of Jamu Samples

Five jamu samples intended for aches, pain, and gout were screened using the developed strips. Based on the result classifications recorded in Table 2, S1 was presumptively positive for dexamethasone only, S2 was presumptively positive for paracetamol only, and S3 and S4 were presumptively positive for both target analytes. Sample S5 was negative for both analytes. Overall, four of the five samples showed a presumptive positive result for at least one target analyte.

Table 2. Colorimetric screening results of jamu samples

Sample code	Analyte tested	Observed color	Recorded result
Negative control	Dexamethasone	Light brown	Negative (-)
	Paracetamol	Orange	Negative (-)
Positive control	Dexamethasone	Brown/light brown	Positive (+)
	Paracetamol	Brown/light brown	Positive (+)
S1	Dexamethasone	Light brown	Positive (+)
	Paracetamol	Orange	Negative (-)
S2	Paracetamol	Dark brown	Positive (+)
	Dexamethasone	Dark brown	Negative (-)
S3	Paracetamol	Light brown	Positive (+)
	Dexamethasone	Dark brown	Positive (+)
S4	Paracetamol	Dark brown	Positive (+)
	Dexamethasone	Light brown	Positive (+)
S5	Paracetamol	Orange	Negative (-)
	Dexamethasone	Dark brown	Negative (-)

Note: Results are reported exactly according to the positive and negative classifications in the original dataset. Positive results are presumptive and require confirmation by a selective instrumental method.

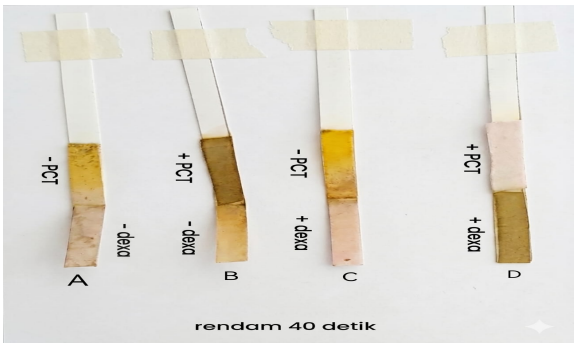

Figure 2. Visual response of the negative and positive reference test strips.

DISCUSSION

1. Interpretation of the Paper-Based Colorimetric Platform

The present findings support the feasibility of a reagent-impregnated paper strip as an initial visual screening platform for suspected chemical drug adulteration in jamu. A visible color response demonstrates that the reagent remains reactive after immobilization on the paper substrate. This approach is consistent with the paper-based paracetamol strip developed by Sentat et al. (2019), which used reagent-impregnated filter paper as a simple screening format for jamu. However, a visible response alone does not establish the identity or concentration of an analyte. The results therefore support preliminary screening rather than definitive qualitative identification or quantitative determination.

The present dataset also does not include a systematic comparison of adsorption time, reagent loading, paper type, or storage conditions. Thus, the study can report that the strips produced visible responses under the applied conditions, but it cannot establish that a specific immersion time was optimal. Future development should compare these variables using the same reference concentration and a predefined color-response criterion.

2. Paracetamol Response with FeCl3

Paracetamol contains a phenolic group that can interact with ferric ions to form a colored complex. In this study, the direct reaction with the paracetamol reference solution produced a yellowish-green response, whereas the immobilized FeCl3 strip was recorded as changing from yellow to orange. A difference between direct-solution and paper-strip color responses can arise from the paper background, reagent concentration on the strip, lighting, drying, and the visual nature of the observation. Because these factors were not separately tested, the recorded strip color should be used as the operational criterion for this study and should be standardized with a color reference chart in later work.

The FeCl3 reaction is useful for rapid screening, but it is not fully selective for paracetamol because herbal matrices can contain phenolic compounds and tannins. Fanani and Yunitasari (2025) likewise combined an FeCl3 color test with thin-layer chromatography when analyzing paracetamol in jamu, and they noted the possibility of matrix interference in interpreting color changes. Accordingly, the presumptive paracetamol-positive classifications in S2, S3, and S4 should not be interpreted as conclusive proof of paracetamol without confirmation by chromatography or another selective technique.

3. Dexamethasone Response with the Acid-Iodine Reagent Mixture

The acid-iodine reagent mixture produced a visible response in the dexamethasone reference test and generated a light-brown to dark-brown strip change in the suitability table. This result indicates that the reagent system can serve as a candidate visual indicator for a dexamethasone-related response. Nevertheless, the data do not demonstrate that the reaction is selective only for dexamethasone. The apparent color can be affected by the complex herbal matrix, the concentration of the analyte, the reagent composition, and the time of observation. Therefore, the dexamethasone-positive results in S1, S3, and S4 should be described as presumptive.

This caution is supported by previous work on dexamethasone detection in herbal products. Pratiwi et al. (2023) developed a polymer-based dexamethasone indicator as a rapid screening tool, while Permatasari et al. (2021) used FTIR and TLC-densitometry to characterize and quantify dexamethasone in rheumatic-pain herbal medicines. These studies show that rapid indicators can support early screening, but orthogonal analytical methods are required when the aim is confirmation or content determination.

4. Interpretation of the Jamu Screening Results

The recorded screening criteria classified four of the five tested samples as presumptively positive for at least one target analyte. Specifically, S1 was classified as dexamethasone-positive, S2 as paracetamol-positive, and S3 and S4 as positive for both analytes. S5 was negative for both analytes. This pattern is relevant because the samples were products marketed for symptoms such as aches, pain, and gout, for which rapid symptom relief may encourage the illicit addition of analgesic or corticosteroid drugs.

The findings should not be interpreted as the prevalence of chemical drug adulteration in all jamu products from Temanggung or Indonesia. The study examined only five samples from one market and used a qualitative visual screening method. Even so, the result identifies samples that warrant confirmatory follow-up. Comparable investigations have used TLC together with validated UV-Vis spectrophotometry or HPLC to substantiate the presence and quantity of paracetamol and dexamethasone in jamu products (Trisiani et al., 2026). Recent national surveillance also continues to identify natural medicine products containing paracetamol and dexamethasone among products claiming relief of pegal linu, underlining the need for routine screening and confirmatory follow-up (Badan Pengawas Obat dan Makanan Republik Indonesia, 2025).

5. Limitations and Analytical Validation Needs

Several limitations should be addressed before the test strips are used for routine monitoring. The study did not evaluate selectivity against common jamu ingredients, visual detection limits, repeatability, intermediate precision, accuracy or recovery, robustness, observer agreement, or storage stability. The study also did not include replicate measurements or a confirmatory instrumental method. In addition, Table 2 contains some identical observed colors assigned to different result categories, which reinforces the need for predefined color criteria and documented interpretation rules.

Future work should test blank jamu matrices spiked with known concentrations of paracetamol and dexamethasone, include positive and negative controls in each run, and assess the effect of extraction solvent, pH, reaction time, light exposure, and storage. A color card or image-based analysis under standardized lighting would reduce subjective interpretation. Positive samples should then be confirmed by TLC-densitometry, HPLC, or LC-MS. The ICH Q2(R2) guideline emphasizes demonstrating that an analytical procedure is fit for its intended purpose through an appropriate validation strategy (International Council for Harmonisation, 2023). Although the present strip is intended for preliminary screening, this principle remains relevant to its development.

CONCLUSION

A colorimetric paper-strip prototype based on Whatman No. 4 filter paper was developed for preliminary screening of paracetamol and dexamethasone in jamu preparations. The FeCl3 strip and the acid-iodine reagent strip produced visible color responses in the suitability assessment. Based on the recorded criteria, four of five jamu samples were presumptively positive for at least one target analyte. The strips are practical, rapid, and economical for early screening; however, they do not provide definitive identification or concentration data. Selectivity testing, analytical validation, standardized color interpretation, and confirmatory instrumental analysis are required before the method is used for routine safety monitoring or regulatory decisions.

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