



RESEARCH ARTICLE

EVALUATION OF YELLOW ASOKA (IXORA COCCINEA L.) FLOWER ETHANOL EXTRACT AS A NATURAL ACID–BASE INDICATOR

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ARTICLE INFO	ABSTRACT
Received: July 1 2026 Accepted: September 21, 2026 Keywords: Yellow Asoka; natural acid–base indicator; pH response; acid–base titration; ethanol extract *Corresponding Author: jusman_palu04@yahoo.com	Natural acid–base indicators derived from flowers offer a potential alternative to synthetic indicators, but their performance depends strongly on extraction solvent and pH response. This study evaluated Yellow Asoka (<i>Ixora coccinea</i> L.) flower extracts prepared with 96% ethanol and ethyl acetate for pH-dependent color behavior and application in acid–base titration. The extracts were examined across buffer solutions from pH 1–13, and the ethanol extract was subsequently tested in strong acid–strong base, weak acid–strong base, and strong acid–weak base titration systems against conventional indicators. Ethanol produced a dark-yellow extract with a 19.77% yield, whereas ethyl acetate produced a lighter-yellow extract with a 6.99% yield. The ethyl acetate extract showed no visible color response across pH 1–13, while the ethanol extract remained colorless at pH 1–6 and changed from yellow to orange at pH 7–13. In hydrochloric acid–sodium hydroxide titration, the ethanol extract changed at 7.4 mL and pH 9.6, compared with 6.6 mL and pH 8.8 for phenolphthalein. For acetic acid–sodium hydroxide, the extract responded at 7.5 mL and pH 10.4, while strong acid–weak base titration produced an endpoint at 7.7 mL and pH 2.16. The findings demonstrate solvent-dependent extraction and pH-responsive indicator behavior, with practical applicability dependent on titration chemistry.

INTRODUCTION

The increasing emphasis on environmentally responsible analytical practices has stimulated renewed interest in natural reagents as alternatives to conventional synthetic chemicals. In acid–base titration, synthetic indicators are widely used because of their defined transition ranges and readily observable color changes; however, the principles of green and sustainable analytical chemistry have encouraged the exploration of plant-derived materials that are renewable, biodegradable, and potentially less dependent on conventional chemical reagents. Recent reviews have highlighted natural plant extracts as promising candidates for green acid–base titrimetric analysis, particularly when extraction procedures and analytical conditions are appropriately controlled (Suteerapataranon et al., 2024; Maqsood et al., 2025). Suteerapataranon et al. (2024) emphasized that the renewed application of natural indicators is closely associated with sustainable analytical chemistry because plant-derived reagents can contribute to reductions in chemical consumption and waste generation. Similarly, Maqsood et al. (2025) reviewed several extraction approaches, including maceration, alcoholic extraction, cold distillation, electrolyte-based extraction, and ultrasonic treatment, and identified anthocyanin-containing plant extracts as particularly relevant to pH-responsive indicator development.

Despite this potential, the development of natural acid–base indicators remains an analytical challenge because the presence of colored phytochemicals does not necessarily guarantee a sufficiently distinct or reproducible indicator response. The suitability of a plant extract is determined by several interrelated characteristics, including pigment composition, pigment concentration, extraction efficiency, solvent selectivity, pH transition interval, solubility, and chemical stability (Maqsood et al., 2025). Evidence from comparative studies illustrates this species-dependent behavior. Samuel et al. (2025), for example, evaluated water-based extracts from 10 plant species and found that only six exhibited visible pH-dependent color changes, while three species produced endpoints particularly close to those obtained with synthetic indicators. The titrant volumes obtained with plant-derived indicators ranged from 4.21 ± 0.06 to 7.60 ± 0.44 mL, demonstrating that plant extracts can generate measurable titrimetric responses but that their analytical suitability cannot be generalized across plant species. This variability establishes the need for systematic evaluation of individual botanical sources rather than assuming that all colored flowers will provide equivalent acid–base indicator performance.

Taken together, the recent literature establishes three important considerations for the development of yellow *I. coccinea* flower extract as an acid–base bioindicator. First, natural indicators are increasingly relevant to sustainable analytical chemistry, but their performance remains strongly dependent on pigment chemistry and extraction conditions (Suteerapataranon et al., 2024; Maqsood et al., 2025). Second, yellow *I. coccinea* flowers contain substantial quantities of flavonoids, anthocyanins, carotenoids, and phenolic compounds, providing a direct phytochemical basis for investigating their color-responsive behavior (Khamla et al., 2024). Third, although *I. coccinea* has already been examined as a natural indicator, the available recent study primarily focused on ethanol extraction and indicator-paper development over pH 1–12 rather than systematically comparing ethanol with ethyl acetate and evaluating the extracts across different titration systems (Riyanti et al., 2022). The species-specific variation demonstrated by Samuel et al. (2025) further supports the need for experimental validation of each plant source, solvent system, and titration application rather than extrapolation from other botanical materials.

Accordingly, this study aims to evaluate yellow *Ixora coccinea* flower extracts prepared using ethanol and ethyl acetate as potential natural acid–base bioindicators by examining their extraction characteristics, pH-dependent color response, and suitability for different acid–base titration systems. The central premise is that differences in solvent polarity and pigment selectivity will influence the composition of the extracted chromophoric compounds and, consequently, their visible response to changes in pH. The study specifically focuses on comparison of ethanol and ethyl acetate extracts, characterization of their color behavior across pH 1–13, and assessment of their applicability in strong acid–strong base, weak acid–strong base, and strong acid–weak base titrations. The novelty lies in integrating solvent-dependent extraction with direct pH-response characterization and titrimetric evaluation of yellow *I. coccinea* flowers, thereby extending previous work that primarily examined ethanol-derived Ashoka indicator materials. The resulting evaluation is expected to clarify the relationship between extraction solvent and indicator performance and to establish an evidence-based basis for determining the analytical applicability of yellow *I. coccinea* flower extract as a natural acid–base indicator.

Literature Review

The analytical potential of plant extracts originates principally from their chemically diverse pigments and their ability to undergo structural or electronic changes in response to environmental conditions. Natural pigments include flavonoids, anthocyanins, carotenoids, betalains, and other chromophoric compounds, with differences in molecular structure influencing their color, absorption characteristics, stability, and environmental responsiveness (Lan et al., 2023; Nabi et al., 2023). Anthocyanins are particularly important because their molecular structures are strongly influenced by solution pH. More than 500 naturally occurring anthocyanins have been reported, and their structural diversity contributes to differences in color expression, stability, and pH sensitivity among plant sources

(Oladzadabbasabadi et al., 2022). Changes in protonation state can modify the equilibrium among different molecular forms, resulting in observable changes in color as pH changes. Kaur et al. (2024) further noted that anthocyanins are generally more stable under acidic conditions, whereas increasing pH promotes structural transformation and can progressively modify their color response. The chromogenic behavior of natural pigments is also influenced by environmental factors such as light, temperature, oxygen, and oxidation, indicating that the performance of a plant-based indicator depends not only on the presence of pigments but also on their chemical stability under the intended analytical conditions (Lan et al., 2023; Nabi et al., 2023).

Within this context, *Ixora coccinea* L. represents a relevant botanical source because the species contains diverse phytochemical constituents associated with its biological and chromatic properties. Shreelakshmi et al. (2021) characterized *I. coccinea* fruits and reported total phenolics of 128 mg/100 g fresh weight, flavonoids of 20.63 mg/100 g, and anthocyanins of approximately 30 mg/100 g, together with identifiable phenolic constituents such as sinapic acid and myricetin. More directly relevant to the present investigation, Khamla et al. (2024) characterized an 80% ethanolic extract of yellow *I. coccinea* flowers and reported flavonoids of 277.38 ± 5.10 mg/g, anthocyanins of 63.71 ± 0.70 mg/g, carotenoids of 74.89 ± 0.72 mg/g, and phenolics of 11.49 ± 4.06 mg/g. The simultaneous presence of flavonoids, anthocyanins, carotenoids, and phenolic compounds indicates that yellow *I. coccinea* flowers contain multiple pigment-related and chromophoric constituents that warrant investigation for pH-responsive applications.

Previous research has provided direct evidence that *I. coccinea* can function as a natural acid–base indicator, although the available investigations have focused on particular extraction systems and indicator formats. Riyanti et al. (2022) investigated *I. coccinea* flower extracts prepared using 96% and 70% ethanol through 24-hour maceration and evaluated their response using buffer solutions covering pH 1–12. The extracted pigment was subsequently incorporated into indicator paper, demonstrating the potential of *I. coccinea* as a natural pH-responsive material. The study therefore establishes an important foundation for the use of *I. coccinea* in acid–base indication, but its emphasis on ethanol extraction and indicator-paper development leaves questions concerning the influence of alternative solvent systems and the direct application of the resulting extracts in titrimetric analysis. This issue is particularly relevant because pigment recovery is strongly affected by solvent properties and extraction conditions.

Solvent selection is a critical factor in obtaining plant pigments because different pigment classes possess different chemical characteristics, polarity, solubility, and stability. Shen et al. (2023) emphasized that extraction procedures can substantially influence pigment recovery and purity, while modern extraction approaches are increasingly evaluated in relation to efficiency, energy consumption, and environmental performance. Kaur et al. (2024) similarly reported that anthocyanin extraction is influenced by solvent type, solvent-to-solid ratio, particle size, temperature, and extraction time; in one reported example, 20% ethanol containing 1% acetic acid yielded 763.80 mg/100 mL anthocyanins from *Syzygium cumini*. These observations demonstrate that solvent composition can affect not only extraction yield but also pigment composition, purity, and stability. Nabi et al. (2023) further emphasized that natural pigments differ in their physicochemical properties and can be sensitive to light, oxygen, and oxidation, reinforcing the importance of matching extraction conditions with the chemical characteristics of the target pigment fraction. Thus, comparison of ethanol and ethyl acetate provides a scientifically relevant approach for examining whether solvent polarity and pigment selectivity influence the observable acid–base response of yellow *I. coccinea* flower extracts.

The analytical application of natural flower extracts has also been demonstrated in several plant systems, supporting the need to evaluate indicator performance under actual titration conditions rather than solely through qualitative color screening. Nsi et al. (2022) evaluated ethanol and hot-water extracts of *Dicliptera verticillata* using UV–visible spectroscopy and four acid–base titration systems, namely strong acid–strong base, strong acid–weak base, weak acid–strong base, and weak acid–weak base. Their extracts generated endpoints comparable with conventional indicators such as methyl orange and phenolphthalein. Kalakonda et al. (2023) likewise reviewed plant-derived indicators and emphasized the contribution of anthocyanins and flavonoids to visible pH-dependent color transitions near titration endpoints. These findings indicate that the suitability of a natural indicator must be assessed according to the specific acid–base system because the equivalence-point pH differs among strong and weak acid–base combinations. For *I. coccinea*, therefore, characterization of the pH response should be complemented by direct evaluation in different titration systems to establish the analytical range in which the extract provides a visually distinguishable endpoint.

RESEARCH METHODOLOGY

2.1 Research Materials and Extraction of Yellow Asoka Flowers

The research used yellow Asoka flowers (*Ixora coccinea* L.) as the botanical source of pigment-based acid–base indicator compounds. Two extraction solvents, 96% ethanol and ethyl acetate, were investigated to examine the influence of solvent characteristics on the resulting flower extracts. The selection of different solvent systems was supported by recent literature indicating that pigment recovery is strongly influenced by solvent composition and extraction conditions. Yan et al. (2023) emphasized that anthocyanin extraction is affected by solvent concentration, material-to-solvent ratio, extraction time, and temperature, whereas Vargas et al. (2024) demonstrated that flower-pigment extraction can be systematically optimized through variation of solvent concentration, temperature, pH,

extraction time, and solid-to-liquid ratio.

The use of ethanol was also relevant to the chemical characteristics of *I. coccinea* flowers. Lertkanchanasak et al. (2024) specifically investigated yellow *I. coccinea* flowers using 80% ethanol followed by concentration with a rotary vacuum evaporator, with the resulting extract subsequently characterized for phenolic, flavonoid, anthocyanin, and carotenoid constituents. This study provides a direct methodological precedent for solvent-based extraction of yellow *I. coccinea*, although the extraction conditions reported in that study were not adopted as experimental parameters for the present work unless explicitly documented in the research record. In the present research, the extraction comparison was specifically performed using 96% ethanol and ethyl acetate. The extraction process and the resulting extracts are documented in Figure 1.



Figure 1. Extraction process of yellow Asoka flowers (*Ixora coccinea* L.) using 96% ethanol and ethyl acetate.

The comparison of ethanol and ethyl acetate was intended to examine solvent-dependent recovery of colored constituents. Recent literature indicates that different pigment classes exhibit different solubility and extraction behavior, and therefore extraction yield does not necessarily represent selective recovery of the particular pigment responsible for indicator activity. The use of alternative solvents is consequently appropriate for evaluating whether the solvent system influences the subsequent pH-responsive behavior of the extract. This consideration is consistent with the methodological framework described by Yan et al. (2023), which emphasizes controlling extraction variables when obtaining and characterizing anthocyanin-containing plant extracts.

2.2 Characterization of pH-Dependent Color Response

The pH response of the extracts was evaluated using buffer solutions covering a broad range from pH 1 to pH 13. The purpose of this evaluation was to determine whether the extracted pigments exhibited observable color changes as a function of acidity or alkalinity and to identify the pH region in which a visually distinguishable transition occurred. This approach follows recent methodological applications in which plant-derived pigments are exposed to sequentially varied pH conditions and their color responses are recorded. Pramitasari et al. (2022), for example, evaluated pigment extracts by mixing 1 mL of extract with 1 mL of buffer solution at pH 1–13 and documenting the resulting color changes.

For the present study, both ethanol and ethyl acetate extracts were examined across the same pH range to permit direct comparison of their visible responses. The visual appearance of each extract under the different pH conditions was documented and used to establish the observable transition region.

Visual assessment was selected as the primary approach because an acid–base indicator must produce a readily observable color transition during analytical use. Nevertheless, recent methodological literature indicates that visual observations can be complemented by instrumental color measurements. Halász et al. (2023) demonstrated the use of CIE Lab* coordinates and total color difference (ΔE) to quantify pH-dependent color changes in plant-pigment systems. Similarly, Yan et al. (2023) described UV–Vis spectroscopy and the differential-pH approach as useful tools for characterizing anthocyanin responses to different pH conditions. In the present research, the pH characterization was based on the documented visual response of the extracts; instrumental color coordinates or spectroscopic measurements were not introduced as experimental parameters because they are not explicitly documented in the research record.

2.3 Application of the Ethanol Extract as an Acid–Base Titration Indicator

Following pH-response characterization, the extract that demonstrated an observable pH-dependent response was evaluated as an indicator in acid–base titration. The ethanol extract of yellow *I. coccinea* was applied to three neutralization systems: strong acid–strong base using HCl–NaOH, weak acid–strong base using acetic acid–NaOH, and strong acid–weak base using HCl–NH₄OH. This experimental arrangement was intended to examine whether the indicator response remained applicable when the equivalence-point conditions differed among acid–base systems.

The methodological approach was based on recent studies demonstrating that natural indicators should be evaluated under actual titration conditions rather than solely through qualitative pH screening. Samuel et al. (2025) evaluated plant extracts through preparation, color-response testing, acid–base titration using natural and synthetic indicators, and subsequent analysis of the titration response. Their study tested multiple plant species and demonstrated variation in endpoint behavior among extracts. Raghavendra et al. (2025) similarly described evaluation across strong acid–strong base, strong acid–weak base, weak acid–strong base, and weak acid–weak base systems as a means of assessing indicator suitability under different titration characteristics.

For each titration system investigated in the present study, the endpoint indicated by the Yellow Asoka ethanol extract was compared with the endpoint obtained using a conventional synthetic indicator. Phenolphthalein was used for the strong acid–strong base and weak acid–strong base systems, whereas methyl orange was used for the strong acid–weak base system. The endpoint was identified from the observable color transition of the indicator-containing solution and documented together with the corresponding titration volume and pH reported in the experimental record.

2.4 Comparison with Conventional Acid–Base Indicators

The comparison with phenolphthalein and methyl orange was performed to establish the relationship between the color transition of the Yellow Asoka extract and the response of established synthetic indicators. The selection of these reference indicators follows the methodological framework summarized by Maqsood et al. (2025), which emphasizes that natural-indicator performance should be evaluated relative to the transition characteristics of conventional indicators and the particular acid–base system under investigation. Phenolphthalein is commonly associated with titration systems requiring an endpoint in the alkaline transition region, whereas methyl orange is applicable to systems in which the endpoint occurs within a more acidic transition interval.

The comparison was based on the observed endpoint color, titrant volume, and pH at the indicator transition. Ghezal et al. (2022) similarly proposed evaluating natural indicators through their color-transition range, pK_a-related behavior, and correspondence with conventional indicators. Samuel et al. (2025) further demonstrated that titrant volume and differences between natural and synthetic indicators can provide quantitative information for assessing indicator performance. Consequently, the present study retained titrant volume and endpoint pH as the principal comparative parameters.

2.5 Experimental Procedure

The experiment was conducted sequentially to evaluate the potential of yellow Asoka (*Ixora coccinea* L.) flower extract as a natural acid–base indicator. Yellow Asoka flowers were extracted separately using 96% ethanol and ethyl acetate. The resulting extracts were collected, and their physical appearance and extraction yields were recorded.

The obtained extracts were then subjected to pH-response testing using buffer solutions covering the pH range of 1–13. Each extract was exposed to the respective pH conditions, and the resulting color changes were observed visually. The extract showing a distinct and observable pH-dependent color response was subsequently selected for evaluation in acid–base titration.

The selected 96% ethanol extract was applied as an indicator in three acid–base titration systems: strong acid–strong base (HCl–NaOH), weak acid–strong base (CH₃COOH–NaOH), and strong acid–weak base (HCl–NH₄OH). During each titration, the solution was observed visually for a distinct color transition, which was used to identify the indicator endpoint. The endpoint obtained using the Yellow Asoka ethanol extract was subsequently compared with the endpoint obtained using the corresponding conventional indicator.

For the strong acid–strong base and weak acid–strong base titration systems, the Yellow Asoka ethanol extract was compared with phenolphthalein. For the strong acid–weak base system, the extract response was compared with methyl orange. The titration response was recorded based on the titrant volume, pH at the observed color transition, and direction of the color change.

Photographs were taken during the experimental procedures to document the observed color changes and experimental conditions. The photographic records were used as supporting visual documentation of the experimental observations and were not treated as independent quantitative measurements.

RESULTS AND DISCUSSION

3.1 Extraction of Yellow Asoka Flowers (*Ixora coccinea* L.)

Yellow Asoka flowers (*Ixora coccinea* L.) were extracted using 96% ethanol and ethyl acetate, producing visibly different extract characteristics. The ethanol extract exhibited a dark-yellow color, whereas the ethyl acetate extract exhibited a lighter-yellow color. The extraction yields were 19.77% for the ethanol extract and 6.99% for the ethyl acetate extract, indicating that ethanol recovered a substantially greater fraction of extractable constituents from the flower material.

The difference in extraction yield is consistent with the physicochemical diversity of plant pigments and other phytochemicals. Plant pigments such as anthocyanins, carotenoids, and betalains differ in polarity, molecular structure, solubility, extraction behavior, and stability; consequently, solvent selection can substantially influence both extraction yield and the composition of the resulting extract (Rodríguez-Mena et al., 2023; Shen et al., 2023).

The higher yield obtained with ethanol is also relevant to the phytochemical composition previously reported for yellow *I. coccinea*. Lertkanchanasak et al. (2024) reported that an 80% ethanolic extract of yellow *I. coccinea* flowers contained phenolics, flavonoids, anthocyanins, and carotenoids. The reported concentrations were 11.49 ± 4.06 mg/g phenolics, 277.38 ± 5.10 mg/g flavonoids, 63.71 ± 0.70 mg/g anthocyanins, and 74.89 ± 0.72 mg/g carotenoids, with flavonoids representing the highest measured phytochemical group. The coexistence of these compound classes provides a relevant chemical background for the different optical characteristics observed between the two solvent extracts.

The influence of solvent and extraction conditions on pigment recovery has also been documented in recent literature. Yan et al. (2023) demonstrated that solvent concentration, solid–liquid ratio, extraction time, and temperature can substantially influence the recovery of anthocyanin-containing extracts. Gwarzo et al. (2023) further demonstrated that solvent extraction can recover pigment constituents from *I. coccinea* flowers that subsequently exhibit acid–base indicator activity.

Table 1. Extraction yield and visual characteristics of yellow Asoka flower extracts

Extraction solvent	Extract color	Extraction yield
Ethanol (96%)	Dark yellow	19.77%
Ethyl acetate	Light yellow	6.99%

The higher yield of the ethanol extract does not by itself establish that ethanol selectively recovered the specific pigment responsible for the acid–base response. Rather, it indicates that ethanol under the conditions used in the present experiment extracted a greater total amount of soluble material. This distinction is important because plant extracts may contain several chemically different pigment and non-pigment constituents, each having different polarity and stability characteristics (Rodríguez-Mena et al., 2023; Shen et al., 2023).

The result is also consistent with the specific use of ethanol for Asoka flower indicator preparation reported by Riyanti et al. (2022). Their study investigated *I. coccinea* flower extract prepared using 96% and 70% ethanol and subsequently evaluated the extracts across pH 1–12. Thus, the present observation of a substantially higher yield from the 96% ethanol system is relevant to the subsequent selection of the ethanol extract for pH-response and titration evaluation.

3.2 Ethyl Acetate Extract pH-Range Test

The ethyl acetate extract was evaluated using buffer solutions covering pH 1–13. No visually observable color change was detected throughout the entire tested pH range. Consequently, the ethyl acetate extract did not demonstrate a distinguishable visual pH response under the experimental conditions used.

The absence of a visible color transition should not necessarily be interpreted as evidence that the extract contained no pigment compounds. The optical response of natural pigments depends on pigment structure, solvent environment, pH, and accessibility of the pigment to the aqueous medium. Rodríguez-Mena et al. (2023) emphasized that solvent compatibility and extraction conditions can influence the observable behavior of plant pigments. Maqsood et al. (2025) similarly noted that natural indicator extracts obtained through different extraction procedures can exhibit different phytochemical compositions and pH-dependent color responses.



Figure 2. pH-response profile of the ethyl acetate extract of yellow Asoka flowers.

The figure shows the appearance of the ethyl acetate extract in buffer solutions from pH 1 to 13, with no observable visual color transition throughout the tested range.

The result differs from the behavior of the ethanol extract described in the following section. This difference indicates that solvent selection was associated not only with extraction yield but also with the subsequent observable pH response. Natural pigment systems can exhibit substantial differences in aqueous behavior depending on the chemical environment surrounding the pigment molecules. Lukitasari et al. (2024), for example, reported pronounced pH-dependent changes in betalain extracts, including spectral changes above pH 8 and degradation under prolonged exposure to strongly alkaline conditions. Wu et al. (2022) likewise reported pH-dependent stability and color behavior in *Bougainvillea* betalain extracts.

Accordingly, the absence of a visible response in the ethyl acetate extract across pH 1–13 represents an experimental distinction from the ethanol extract rather than evidence that solvent polarity alone determines pigment behavior. The result supports the subsequent selection of the ethanol extract for evaluation as a natural acid–base indicator.

3.3 Ethanol Extract pH-Range Test

The ethanol extract exhibited a substantially different response when evaluated across the same pH 1–13 range. The extract remained colorless at pH 1–6, whereas a distinct transition from yellow to orange occurred at pH 7–13. The appearance of different colors at different pH regions demonstrates that the ethanol extract possesses measurable pH-dependent optical behavior.

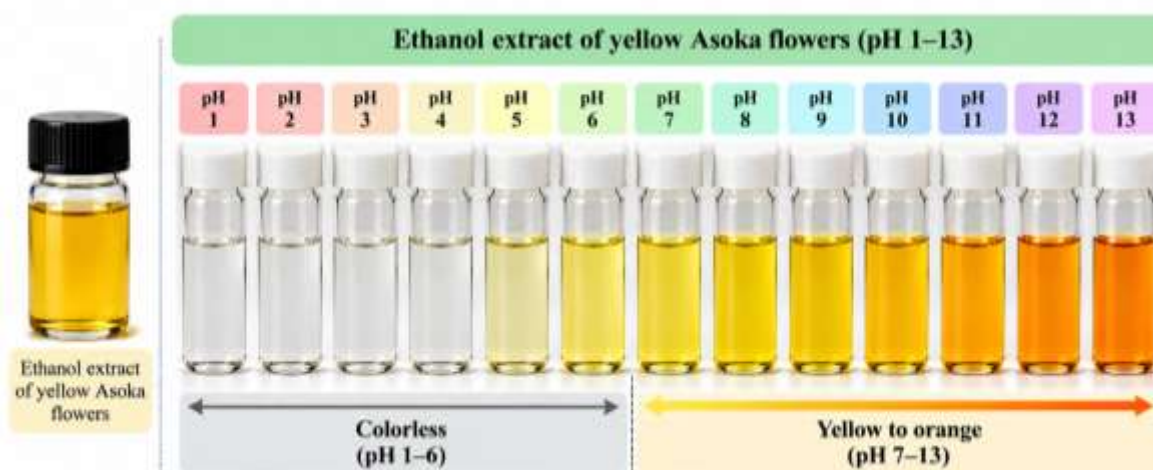


Figure 3. pH-response profile of the ethanol extract of yellow Asoka flowers.

The figure illustrates the pH-dependent color response of the ethanol extract, showing a colorless appearance at pH 1–6 and a yellow-to-orange transition at pH 7–13.

The observed response is consistent with recent research showing that plant-derived pigments can undergo structural and optical changes as a function of pH. Nag et al. (2023) described the pH-dependent behavior of natural pigments and emphasized that protonation and deprotonation processes can alter the molecular forms responsible for visible color. Rahmawati et al. (2024) similarly demonstrated that flower extracts can exhibit distinct and visually recognizable colors over different acidic, neutral, and alkaline pH regions.

The present result is particularly relevant because previous research has independently demonstrated the indicator potential of *I. coccinea*. Riyanti et al. (2022) reported that 96% ethanolic Asoka flower extracts could be calibrated over pH 1–12 and exhibited progressive color changes with increasing pH. The present experiment extends this observation to a pH range of 1–13 and demonstrates a distinct color response from colorless under acidic conditions to yellow-orange under neutral-to-alkaline conditions.

The pH-dependent response can also be considered in relation to the phytochemical composition of yellow *I. coccinea*. Lertkanchanasak et al. (2024) identified flavonoids, anthocyanins, carotenoids, and phenolic compounds in yellow *I. coccinea* flower extract. These compounds have different chemical structures and optical characteristics, and their combined presence may contribute to the overall color observed in the crude extract. However, the present results do not establish which individual compound or compound class is responsible for the yellow-to-orange transition.

The observed pH response is therefore interpreted as a property of the whole ethanol extract, rather than being assigned to a single pigment without additional chromatographic or spectroscopic identification. This interpretation is consistent with Maqsood et al. (2025), who emphasized that natural-indicator performance depends on extract composition, extraction method, pigment response to pH, and compatibility between the transition region and the titration system.

3.4 Application of Yellow Asoka Flower Extract as a Titration Indicator

Because the ethanol extract exhibited a clear pH-dependent response whereas the ethyl acetate extract did not, the ethanol extract was subsequently evaluated in acid–base titration systems. Three titration combinations were examined: strong acid–strong base (HCl–NaOH), weak acid–strong base (acetic acid–NaOH), and strong acid–weak base (HCl–NH₄OH). The results demonstrate that the endpoint response of the Yellow Asoka extract differed according to the titration system.

Table 2. Titration response of Yellow Asoka ethanol extract and conventional indicators

Titration system	Indicator	Titrant volume	Endpoint pH	Color change
Strong acid–strong base (HCl–NaOH)	Phenolphthalein	6.6 mL	8.8	Colorless → pink
Strong acid–strong base (HCl–NaOH)	Yellow Asoka ethanol extract	7.4 mL	9.6	Colorless → yellow
Weak acid–strong base (acetic acid–NaOH)	Phenolphthalein	7.0 mL	9.1	Colorless → pink
Weak acid–strong base (acetic acid–NaOH)	Yellow Asoka ethanol extract	7.5 mL	10.4	Colorless → yellow
Strong acid–weak base (HCl–NH ₄ OH)	Yellow Asoka ethanol extract	7.7 mL	2.16	Yellow → colorless
Strong acid–weak base (HCl–NH ₄ OH)	Methyl orange	6.35 mL	3.3	—

3.4.1 Strong Acid–Strong Base Titration

In the HCl–NaOH titration, phenolphthalein indicated the endpoint at 6.6 mL and pH 8.8, with a color transition from colorless to pink. In comparison, the Yellow Asoka ethanol extract indicated the endpoint at 7.4 mL and pH 9.6, accompanied by a transition from colorless to yellow.

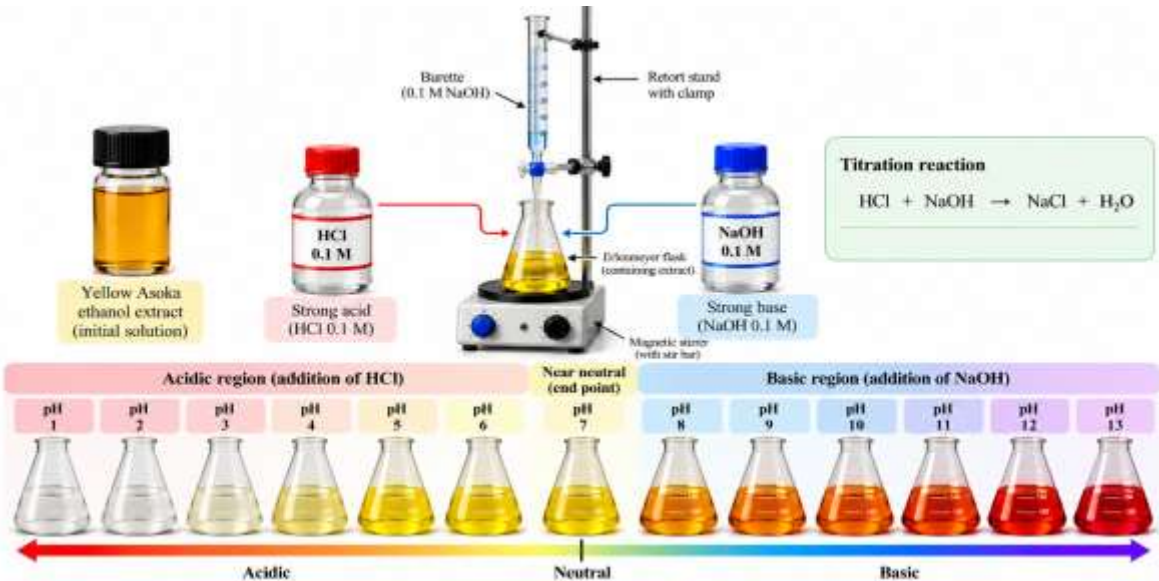


Figure 4. Change in colour of the solution using the Yellow Asoka ethanol extract after titration of strong acid with strong base

The Yellow Asoka extract therefore exhibited an alkaline-region response in this titration system. This finding is compatible with the broader evidence that *I. coccinea* pigments can function as acid–base indicators. Gwarzo et al. (2023) evaluated *I. coccinea* flower extract in strong acid–strong base, strong acid–weak base, and weak acid–strong base titrations and reported measurable endpoint responses for the extract.

More recently, Raghavendra et al. (2025) reported that plant extracts, including *I. coccinea* flowers, could produce titration endpoints comparable with conventional indicators, with endpoint errors relative to synthetic indicators ranging from 1.58% to 4.54%. These findings support the use of endpoint deviation and transition-region compatibility as quantitative criteria for evaluating natural indicators.

3.4.2 Weak Acid–Strong Base Titration

In the acetic acid–NaOH system, phenolphthalein indicated the endpoint at 7.0 mL and pH 9.1, accompanied by a color change from colorless to pink. The Yellow Asoka ethanol extract indicated the endpoint at 7.5 mL and pH 10.4, with the solution changing from colorless to yellow.

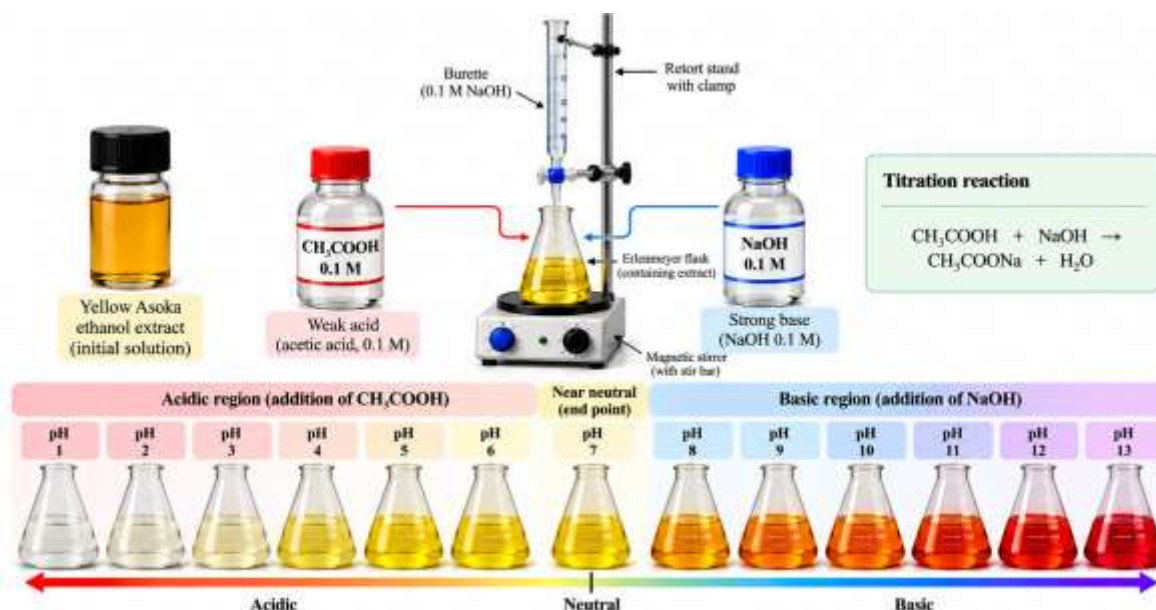


Figure 5. Change in colour of the solution using the Yellow Asoka ethanol extract after titration of weak acid with strong base.

The Yellow Asoka extract therefore showed an endpoint response at a higher pH than phenolphthalein under this experimental condition. The difference illustrates the importance of matching the transition behavior of a natural indicator with the pH region surrounding the equivalence point. Nag et al. (2023) emphasized that indicator suitability depends on whether the color-transition interval overlaps the rapid pH change occurring near the equivalence point.

Recent work with other flower extracts similarly demonstrates that natural-indicator transition intervals may vary among titration systems. Rahmawati et al. (2024), for example, reported a distinct transition behavior for *Torenia fournieri* in weak-acid–strong-base titration and emphasized the importance of comparing the natural extract with conventional indicators. Samuel et al. (2025) likewise demonstrated that natural indicators with perceptible color transitions can produce equivalence points closely aligned with synthetic indicators, although their analytical performance varies among plant extracts and titration systems.

3.4.3 Strong Acid–Weak Base Titration

In the HCl–NH₄OH system, the Yellow Asoka ethanol extract indicated the endpoint at 7.7 mL and pH 2.16, with a color transition from yellow to colorless. Methyl orange, used as the conventional reference indicator, indicated the endpoint at 6.35 mL and pH 3.3. The reported methyl-orange transition range was pH 3.0–4.6.

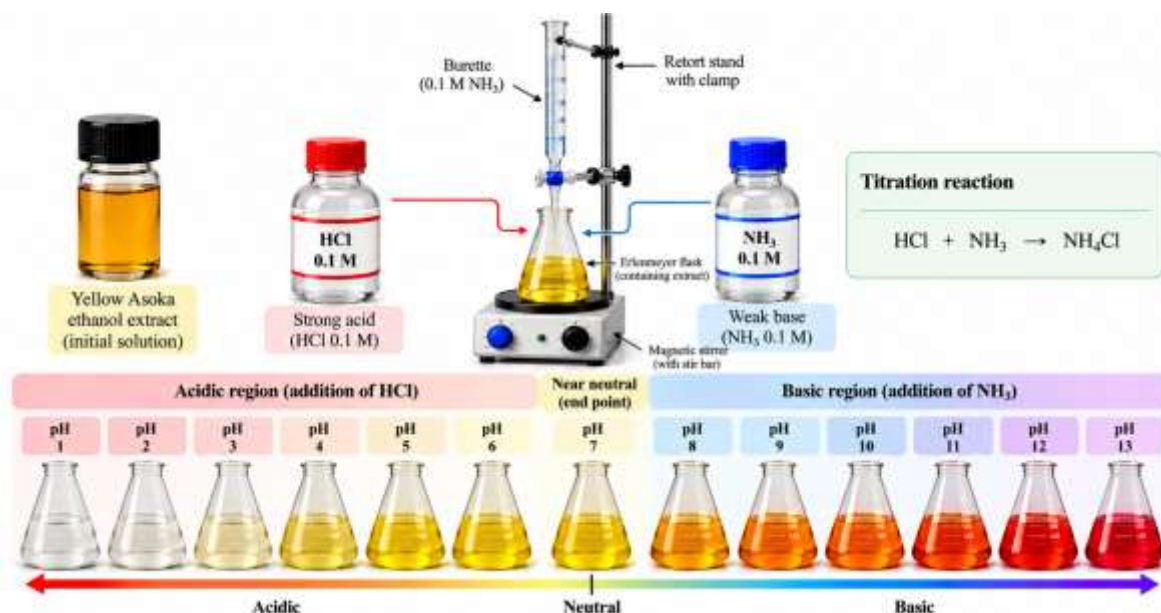


Figure 6. Change in colour of the solution using the Yellow Asoka ethanol extract after titration of strong acid with weak base.

The Yellow Asoka extract therefore exhibited an endpoint response at a lower pH than the methyl-orange reference point. The difference indicates that the color-transition region of the crude ethanol extract did not coincide completely with the reference indicator response in this titration system.

This observation should be interpreted in the context of previous studies on *I. coccinea*. Gwarzo et al. (2023) specifically evaluated *I. coccinea* flower extract in a strong-acid–weak-base system and reported no appreciable difference between the mean titre obtained using methyl orange and the *Ixora* extract. The difference between the published result and the present endpoint behavior indicates that natural-indicator performance can depend on extraction procedure, extract composition, experimental conditions, and the specific endpoint criterion used.

Raghavendra et al. (2025) similarly emphasized quantitative comparison of natural and synthetic indicators through endpoint deviation, reporting errors of 1.58–4.54% for investigated plant extracts. Maqsood et al. (2025) further identified extract composition, extraction method, pigment response to pH, and compatibility between the transition range and equivalence-point pH as important determinants of natural-indicator performance.

Taken together, the results demonstrate that the 96% ethanol extract of yellow Asoka flowers exhibited a clearer pH-dependent response than the ethyl acetate extract and could function as a visual acid–base indicator. The ethanol extract changed from colorless at pH 1–6 to yellow–orange at pH 7–13 and subsequently produced observable endpoint responses in all three investigated titration systems. The corresponding endpoint observations were 7.4 mL at pH 9.6 for HCl–NaOH, 7.5 mL at pH 10.4 for acetic acid–NaOH, and 7.7 mL at pH 2.16 for HCl–NH₄OH.

The results also demonstrate that the performance of the Yellow Asoka extract was titration-system dependent. Its response in the strong-acid–strong-base system occurred in an alkaline region close to the conventional phenolphthalein domain, whereas its response in the weak-acid–strong-base system occurred at a higher pH. In the strong-acid–weak-base system, the observed endpoint occurred below the reported methyl-orange transition range. Such system-dependent behavior is consistent with recent literature emphasizing that natural indicators should be evaluated according to the relationship between their color-transition region and the equivalence-point pH rather than being treated as universal substitutes for synthetic indicators (Nag et al., 2023; Maqsood et al., 2025; Raghavendra et al., 2025).

DISCUSSION

4.1 Extraction Yield and Solvent Effects

The extraction results demonstrate a pronounced solvent-dependent difference in the recovery of compounds from yellow *Ixora coccinea* L. flowers. As shown in Figure 1 and Table 1, 96% ethanol produced a dark-yellow extract with a yield of 19.77%, whereas ethyl acetate produced a lighter-yellow extract with a substantially lower yield of 6.99%. This difference is consistent with the established influence of solvent polarity on extraction selectivity. However, extraction yield should not be interpreted solely as an indicator of the recovery of the target pigment. Yewale et al. (2022) demonstrated that solvents producing higher total extract yields do not necessarily provide the highest recovery of particular flavonoid constituents, indicating that total yield and target-compound recovery represent different analytical parameters.

The present result is also consistent with studies specifically examining *Ixora* extracts. Rajayan et al. (2024) reported different extraction profiles for *Ixora* flowers depending on solvent, with ethyl acetate and hydroalcoholic extraction producing distinct yields and flavonoid characteristics. Lertkanchanasak et al. (2024) similarly reported

that yellow *I. coccinea* flowers extracted with 80% ethanol contained substantial quantities of flavonoids, together with phenolics, anthocyanins, and carotenoids. Thus, the higher yield obtained with ethanol in the present study is more appropriately interpreted as evidence of greater recovery of ethanol-soluble constituents rather than proof that ethanol selectively extracted the specific compound responsible for indicator activity.

The different extract colors further support the occurrence of solvent-dependent compositional differences. Rodríguez-Mena et al. (2023) noted that plant pigments possess different chemical structures and extraction characteristics, so changes in solvent can alter both extraction efficiency and pigment composition. Airouyuwa et al. (2024) likewise emphasized that solvent polarity, pH, and other physicochemical properties can influence the recovery of anthocyanins, carotenoids, and xanthophylls. Therefore, the dark-yellow appearance of the ethanol extract and the lighter-yellow appearance of the ethyl acetate extract likely reflect differences in the groups and relative concentrations of compounds transferred into each solvent.

4.2 Ethyl Acetate Extract and pH Response

The absence of a visible color transition in the ethyl acetate extract throughout pH 1–13 (Figure 2) contrasts strongly with the behavior of the ethanol extract. This finding suggests that extraction yield alone cannot predict suitability as a natural acid–base indicator. Although the ethyl acetate extract contained visually detectable yellow compounds, these constituents did not produce an observable pH-dependent response under the experimental conditions.

A plausible interpretation is that solvent–pigment compatibility influenced the accessibility and optical behavior of the extracted compounds in the aqueous buffer system. Rodríguez-Mena et al. (2023) emphasized that the observable properties of natural pigments depend on pigment structure, solvent environment, pH, and extraction conditions. Maqsood et al. (2025) similarly identified extraction method, extract composition, and pigment response to pH as important factors controlling natural-indicator performance.

The result should not, however, be interpreted as evidence that ethyl acetate is intrinsically unsuitable for extracting indicator-active compounds. Rajayan et al. (2024) demonstrated that ethyl acetate extraction of *I. coccinea* flowers can selectively recover flavonoid-rich fractions. The present finding instead indicates that the particular ethyl acetate extract obtained under the experimental conditions did not exhibit sufficient visible pH responsiveness when exposed to aqueous buffers. This distinction is important because natural-pigment behavior is determined by both chemical composition and the environment in which the pigment is evaluated.

4.3 Ethanol Extract and pH-Dependent Color Change

In contrast, the ethanol extract showed a clear pH-dependent response, remaining colorless at pH 1–6 and changing from yellow to orange at pH 7–13 (Figure 3). This transition demonstrates that the ethanol extract contains constituents whose optical properties are sensitive to the acid–base environment. Similar pH-dependent responses have been reported for other natural pigment systems, although the direction and magnitude of color change depend on pigment structure and composition.

Lan et al. (2023) explained that chromophore structure and its chemical environment are central to the color response of natural pigments. pH-induced structural changes can alter the electronic properties of pigment molecules and consequently their visible color. Yan et al. (2023) likewise emphasized that pigment systems, particularly anthocyanins, are sensitive to pH and that changes in pH can induce structural and spectral transformations. Tang et al. (2023) further described the relationship between pH-dependent structural forms and changes in anthocyanin coloration.

Nevertheless, the yellow-to-orange transition observed here should not be attributed specifically to anthocyanins without direct chemical characterization. Lertkanchanasak et al. (2024) demonstrated that yellow *I. coccinea* flowers contain several pigment-related constituents, including flavonoids, anthocyanins, and carotenoids. The observed color response may therefore reflect the combined behavior of several constituents rather than a single pigment. This interpretation is further supported by Wijesekara and Xu (2024), who emphasized that natural-pigment stability and coloration can be affected simultaneously by molecular structure, pH, temperature, light, oxygen, metal ions, and intermolecular interactions.

The broad pH response observed in the present study also warrants consideration of pigment stability. Lukitasari et al. (2024) reported pronounced pH-dependent spectral changes in betalain extracts and substantial degradation under prolonged alkaline exposure. Thus, the visible transition of the Yellow Asoka extract across pH 7–13 demonstrates pH responsiveness, but it does not by itself establish long-term pigment stability throughout the entire alkaline range.

4.4 Application in Acid–Base Titrations

4.4.1 Strong Acid–Strong Base

The HCl–NaOH results provide the clearest correspondence between the Yellow Asoka extract and the conventional indicator. Phenolphthalein produced an endpoint at 6.6 mL and pH 8.8, whereas the Yellow Asoka extract produced an endpoint at 7.4 mL and pH 9.6. The Yellow Asoka response therefore occurred within the alkaline region associated with the reported phenolphthalein transition range in the present dataset.

This behavior is consistent with recent evidence that natural flower extracts can provide visually detectable endpoints in strong acid–strong base systems. Fitri and Fikroh (2021) reported the acid–base indicator potential of *Clitoria ternatea* extract, while Gwarzo et al. (2023) directly demonstrated the application of *I. coccinea* flower pigments in acid–base titration. Raghavendra et al. (2025) further showed that *I. coccinea*-containing plant extracts could be quantitatively compared with phenolphthalein, with reported endpoint errors of 1.58–4.54%. The present difference in titrant volume should therefore be considered as an endpoint-response difference rather than evidence of identical analytical performance.

4.4.2 Weak Acid–Strong Base

The difference became more pronounced in the acetic acid–NaOH system. Phenolphthalein indicated an endpoint at 7.0 mL and pH 9.1, whereas the Yellow Asoka extract produced an endpoint at 7.5 mL and pH 10.4. The extract response therefore occurred above the phenolphthalein range reported in the present manuscript. This displacement indicates that the transition behavior of the Yellow Asoka extract was less closely aligned with the conventional indicator under this titration condition.

The finding illustrates why natural indicators cannot be evaluated independently of the titration system. Rahmawati et al. (2024) reported different transition ranges for *Torenia fournieri* according to the titration system, while Samuel et al. (2025) found that only some plant extracts produced endpoints closely aligned with synthetic indicators. Therefore, the pH-transition interval of the Yellow Asoka extract should be considered an intrinsic analytical characteristic that determines where it can provide useful endpoint detection.

4.4.3 Strong Acid–Weak Base

The HCl–NH₄OH system produced the greatest difference from the conventional reference. The Yellow Asoka extract changed from yellow to colorless at 7.7 mL and pH 2.16, whereas methyl orange indicated the endpoint at 6.35 mL and pH 3.3. The observed Yellow Asoka endpoint was consequently below the reported methyl-orange transition range of pH 3.0–4.6.

This result contrasts with Gwarzo et al. (2023), who reported no appreciable difference between *I. coccinea* extract and methyl orange in a strong-acid–weak-base system. The discrepancy is scientifically relevant because it demonstrates that indicator performance may change with extraction conditions, extract composition, concentration, and endpoint-identification criteria. Rahmawati et al. (2024) similarly showed that natural-indicator transition intervals can differ substantially from those of conventional indicators, reinforcing the need for system-specific validation.

4.5 Overall Interpretation and Application

The experimental pattern indicates that the analytical behavior of Yellow Asoka extract is governed by the interaction between solvent selection, extract composition, pH-dependent pigment behavior, and titration-system requirements. The substantially higher ethanol yield does not necessarily signify greater recovery of a single indicator-active pigment; rather, it indicates greater recovery of ethanol-soluble constituents. The subsequent absence of a visible pH response from the ethyl acetate extract and the pronounced response of the ethanol extract demonstrate that the chemical composition and aqueous compatibility of the extract are more relevant to indicator functionality than extraction yield alone.

From an application perspective, the ethanol extract provides a visually detectable response particularly within the alkaline region and produces observable endpoints in different titration systems. However, the differences between its endpoints and those of phenolphthalein or methyl orange demonstrate that visible color change should not automatically be equated with analytical equivalence. Maqsood et al. (2025) emphasized that natural-indicator performance depends on chemical composition, extraction methodology, pH response, and correspondence between the indicator transition interval and equivalence-point pH. Samuel et al. (2025) and Raghavendra et al. (2025) similarly support quantitative endpoint comparison as a means of evaluating natural-indicator performance.

Further development of Yellow Asoka as a natural indicator would therefore benefit from systematic optimization of extraction parameters and chemical characterization of the resulting extracts. Yan et al. (2023) identified solvent concentration, solid–liquid ratio, extraction time, and temperature as important variables in pigment extraction. Maqsood et al. (2025) additionally highlighted UV–Vis and FT-IR characterization as useful approaches for relating extract composition to indicator behavior. Such characterization would help distinguish whether the observed yellow-to-orange response originates predominantly from flavonoids, anthocyanins, carotenoids, or interactions among multiple pigment constituents, while stability assessment would be necessary to establish reproducibility under different storage and titration conditions.

CONCLUSION AND RECOMMENDATIONS

The results establish that Yellow Asoka flower extract exhibits indicator behavior that is strongly dependent on extraction solvent and titration conditions. Extraction with 96% ethanol produced a dark-yellow extract with a yield of 19.77%, substantially higher than the 6.99% yield obtained with ethyl acetate. The contrasting pH responses further demonstrate the importance of solvent selection: the ethyl acetate extract showed no visible color change from pH 1–13, whereas the ethanol extract displayed a distinct transition from colorless at pH 1–6 to yellow–orange at pH 7–

13. This response enabled the ethanol extract to function as a visual indicator in acid–base titration, although its performance varied among titration systems. In the strong acid–strong base system, the observed endpoint at pH 9.6 was close to the phenolphthalein response at pH 8.8. The weak acid–strong base system produced a higher endpoint pH of 10.4, while the strong acid–weak base system produced an endpoint at pH 2.16, demonstrating that indicator suitability is governed by the correspondence between its color-transition behavior and the chemistry of the titration system. These findings contribute to the body of knowledge on plant-based acid–base indicators by demonstrating the functional relevance of solvent selection and pH-dependent color response in Yellow Asoka flowers.

Recommendations

Further research should characterize the responsible pigment compounds, quantify their pH-dependent spectral changes, and optimize extraction, stability, concentration, and reproducibility to establish reliable analytical performance.

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