



DOI: <https://doi.org>

International Journal of Advanced and Innovative Research
Journal homepage : <https://scholarclub.org/index.php/IJAIR/login>



A Low-Cost Paper-Based Colorimetric Strip for Rapid and On-Site Detection of Phytochemicals

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ARTICLE INFO:

ABSTRACT

Received:

10 06 2026

Revised:

30 07 2026

Accepted:

28 08 2026

Keywords:

phytochemicals; paper-based colorimetric strips; tannins; flavonoids; saponins; alkaloids; phenols; antioxidant activity

*Background: Phytochemicals are plant-derived compounds widely investigated in pharmaceutical development, food-quality assessment, and medicinal-plant research. Conventional qualitative screening is useful but is commonly performed in separate test tubes and can be laborious when multiple samples are evaluated. This study developed a simple paper-based colorimetric strip and compared its qualitative responses with the corresponding conventional procedures. Methods: Wax-defined reaction zones were prepared on chromatography paper and used for qualitative detection of tannins, carbohydrates, alkaloids, flavonoids, saponins, anthraquinones, steroids, glycosides, reducing sugars, and volatile oils in selected medicinal-plant extracts. The strip classifications were compared directly with standard procedures. Additional analyses included extraction yield, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity using FRAP, ABTS, thiocyanate, and DPPH assays. Results: For the samples evaluated, the paper strips reproduced the positive or negative classifications obtained with the corresponding conventional procedures. In *Limnophila aromatica*, extraction yield was highest with 100% methanol ($26.06 \pm 1.64\%$), whereas TPC and TFC were highest with 100% ethanol (40.50 ± 0.88 mg GAE/g and 31.11 ± 0.43 mg QCE/g, respectively). TPC and TFC were strongly correlated ($R = 0.923$). The tested medicinal-plant extracts showed solvent-dependent FRAP and ABTS responses, and*

aqueous Cetraria islandica showed strong antioxidant activity in the thiocyanate and DPPH assays. Conclusion: The paper-based format provides a rapid, inexpensive, and visually interpretable approach for qualitative phytochemical screening. The findings support further optimization of reagent stability, standardized color interpretation, analytical validation, and image-assisted readout before wider routine application.

INTRODUCTION

Phytochemicals are naturally occurring plant constituents that contribute to the color, flavor, aroma, and biological properties of many plant-derived foods and medicinal materials. They include structurally diverse groups such as polyphenols, flavonoids, tannins, alkaloids, saponins, glycosides, terpenoid-related compounds, and other secondary metabolites. Although phytochemicals are not generally classified as essential nutrients, they have attracted sustained research interest because many of them participate in plant defense and exhibit biological activities relevant to human health. Reviews of plant-food phytochemistry and secondary-metabolite biology have emphasized that their effects depend on chemical structure, concentration, bioavailability, and biological context rather than on the simple presence of a compound class [1-3]. Several phytochemical groups have also been investigated as potential contributors to cancer-preventive or health-promoting mechanisms, although such biological potential does not by itself establish clinical efficacy [4].

Medicinal plants remain important sources of compounds used in traditional preparations and in contemporary natural-product research. Their phytochemical composition is influenced by species, plant part, environmental conditions, extraction solvent, temperature, and processing history. Qualitative screening is therefore commonly used as an initial step to determine whether broad classes such as tannins, carbohydrates, alkaloids, flavonoids, saponins, anthraquinones, steroids, glycosides, reducing sugars, and volatile oils are detectable in an extract. Such tests are useful for preliminary characterization and for prioritizing samples for more specific analytical work. Tannins, for example, have a long history of technological and biomedical investigation, including applications associated with their protein-binding, antioxidant, and antimicrobial properties [6]. More broadly, studies of traditional medicinal plants illustrate the continuing use of preliminary phytochemical analysis to connect ethnobotanical material with subsequent laboratory evaluation [5].

Conventional phytochemical tests are usually performed in test tubes by adding a specific reagent and interpreting a precipitate or color change. These procedures are accessible, but processing many samples separately can consume reagents, increase handling steps, and make side-by-side visual comparison less convenient. Paper-based analytical formats offer an alternative because porous cellulose can support small reaction volumes within compact, disposable test zones. Colorimetric paper devices can be designed so that the analytical response is visible without complex instrumentation, while hydrophobic barriers can restrict liquid spreading and enable several reactions on a single strip. Reviews of microfluidic and paper-based colorimetric devices have highlighted their low material requirement, portability, rapid response, and suitability for multiplexed visual testing, while also noting challenges involving reagent storage, uniformity, quantitative readout, and color interpretation [7,8]. These features make paper an attractive substrate for preliminary phytochemical screening, particularly when the intended output is a qualitative positive or negative classification.

Phytochemical screening is often accompanied by measurements of extraction yield, total phenolic content, total flavonoid content, and antioxidant activity because these measurements provide complementary information about the chemical composition of plant extracts. Solvent polarity can substantially influence the recovery of phenolic and other constituents, so comparisons among water, methanol, ethanol, and acetone can produce different extract yields and different apparent phenolic or flavonoid concentrations [9]. Antioxidant activity is likewise method-dependent. The FRAP assay evaluates ferric reducing capacity, whereas ABTS and DPPH assays assess radical-scavenging behavior through different reaction chemistries [10-13]. For that reason, the use of more than one antioxidant assay can provide a broader description of extract behavior than a single test alone.

The present work combines these two analytical levels. First, it evaluates a paper-based colorimetric strip as a simple platform for conventional qualitative phytochemical reactions. Second, it retains the complementary extraction, TPC, TFC, and antioxidant measurements used to characterize selected medicinal-plant extracts. The paper strip was constructed by drawing 1 × 1 cm reaction zones on chromatography paper and surrounding the zones with wax to limit lateral diffusion. The same qualitative reagent systems used in the conventional procedures were then applied on paper, allowing direct comparison between the standard tube result and the strip response for the samples tested. The approach was intentionally designed as a visually interpreted qualitative screen rather than as a fully quantitative sensor.

Accordingly, the primary objective of this study was to develop a low-cost paper-based colorimetric strip for rapid qualitative detection of major phytochemical classes in medicinal-plant extracts and to determine whether the strip classifications agreed with the corresponding conventional tests. A secondary objective was to characterize selected extracts using extraction yield, TPC, TFC, FRAP, ABTS, thiocyanate, and DPPH measurements. By focusing on a simple reaction-zone design and direct visual readout, the study addresses the practical need for a compact format that can accommodate several samples or reactions simultaneously while preserving the chemistry of established qualitative tests. The work is presented as an early-stage analytical development study, and the strip is therefore evaluated as a screening tool rather than as a clinically validated diagnostic device.

A useful analytical workflow separates preliminary screening from compound-specific identification. Qualitative reactions can rapidly indicate whether a broad phytochemical class is detectable, but they do not identify individual molecules or establish concentration with the specificity of chromatographic, spectrometric, or spectroscopic techniques. The purpose of a paper strip in this context is therefore not to replace confirmatory instrumentation. Rather, it can function as an early decision tool that reduces the number of samples requiring more resource-intensive follow-up. This distinction is especially relevant for medicinal-plant studies in which many extracts or fractions may need to be compared before detailed chemical analysis. By transferring established tube reactions to a patterned cellulose support, the present approach retains familiar reagent chemistry while reducing reaction volume and allowing several tests to be viewed side by side. The value of the format depends on whether the paper response remains sufficiently clear to reproduce the qualitative interpretation of the conventional test, which is the central comparison examined in this study.

The reliability of a paper-based color reaction also depends on practical variables that are less prominent in a test tube. Liquid spreading, local reagent concentration, paper thickness, drying time, extract color, storage conditions, and environmental humidity can influence the appearance of the reaction zone. Hydrophobic patterning is therefore important because it confines the liquid to a defined area and limits cross-contamination between neighboring tests. Similar considerations are recognized across paper-based analytical devices, where simple visual readout is balanced against the need for reproducible reagent deposition and controlled interpretation [7,8]. In parallel, the chemical composition of the extract itself depends on the extraction system. Differences in solvent polarity can change the recovery of phenolic and flavonoid constituents [9], and antioxidant measurements can vary because FRAP, ABTS, and DPPH assays respond through different reaction mechanisms [10-13]. For these reasons, the qualitative strip comparison and the complementary TPC, TFC, and antioxidant measurements address related but distinct questions: the first evaluates transfer of conventional phytochemical reactions to paper, whereas the second characterizes selected extracts under the analytical conditions used.

MATERIALS AND METHODS

Reagents and chemicals

Analytical-grade reagents were used throughout the study. Reagents and standards included Gram's iodine (Diachem Diagnostic Division), sodium hydroxide, sulfuric acid (Sigma-Aldrich), ferric chloride [$\text{FeCl}_3(\text{H}_2\text{O})_x$; Sigma-Aldrich], Folin-Ciocalteu (FC) reagent, potassium carbonate, sodium nitrite, the metal-chloride reagent specified in the source protocol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), quercetin, trichloroacetic acid (TCA), sodium ferrous cyanide, FeSO_4 , citric acid, 2-thiobarbituric acid (TBA), nitro blue tetrazolium (NBT), folic acid, magnesium chloride, gallic acid, rutin, 2-deoxy-2-ribose, EDTA, Molisch reagent, Fehling solution, and the stated thiocyanate reagent. Distilled water and the solvents ethanol, methanol, chloroform, acetone, hexane, petroleum ether, and ethyl acetate were also used. Where supplier availability was limited, solvents were prepared in-house following the cited procedures: methanol from syngas, ethanol from glucose fermentation, chloroform according to the stated chlorination/cumene procedure, hexane and petroleum ether by distillation, and ethyl acetate by esterification.

Preparation of paper-based colorimetric strips

Clean chromatography paper was marked with consecutive 1×1 cm squares using a pencil. Wax crayon was applied around each square to create a hydrophobic barrier and define a 1×1 cm reaction zone, thereby limiting diffusion of reagents and extracts outside the test area. The paper was cut into strips containing four reaction zones. For each phytochemical, paired reaction zones were used for the relevant reagent and extract concentrations, and the required tests were performed on the paper-based strips.

Plant materials listed in Table 1 were selected on the basis of their reported medicinal properties. Samples were shade-dried and ground to a coarse powder using a mechanical grinder. For aqueous extraction, plant powder was mixed with 200 mL of distilled water, stirred for 1 h at 70-75 °C, filtered, and centrifuged at 4000 rpm for 15 min. The pellet was discarded and the supernatant retained. After centrifugation, the extract was filtered twice through Whatman Grade 1 qualitative filter paper and stored in a freezer until testing. For the stability assessment, prepared paper-based colorimetric strips were stored at room temperature in closed, labeled polyethylene bags in a drawer and monitored for up to 2 months.

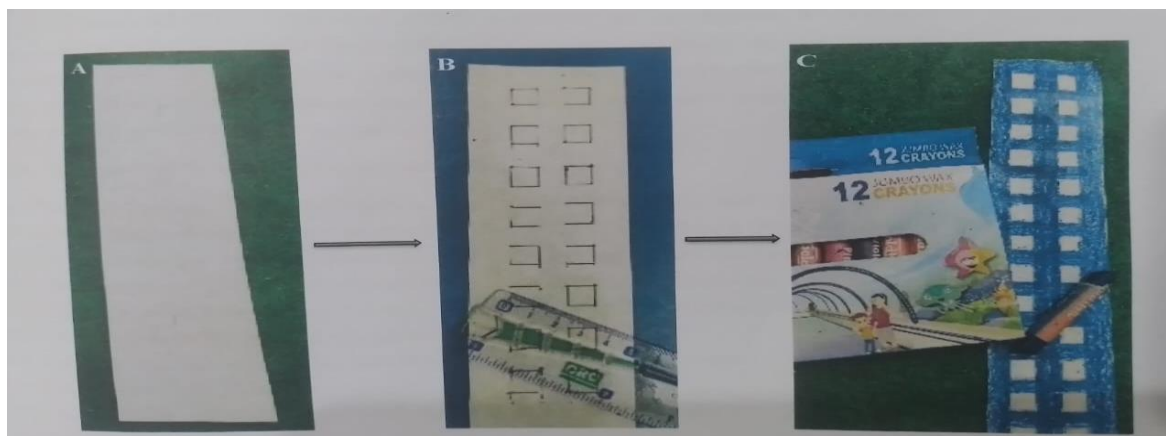


Figure 1. Preparation of the paper-based colorimetric strip.

Plant material and extract preparation

Plant selection was based on medicinal characteristics (Table 1). Leaves and green branches, as applicable, were washed with distilled water and shade-dried for several days. The dried material was ground to a fine powder and labeled (e.g., SP1, SP2). For each sample, 20 g of powder was combined with 200 mL of distilled water in a flask and stirred on a hot plate with a magnetic stirrer for 1 h at 70-75 °C. The mixture was then transferred through muslin cloth into a beaker. The study included 23 identified plant species (Table 1), selected to represent medicinal plants with previously reported or less-characterized phytochemical profiles.

Table 1. Medicinal plant materials used in the study

Sample	Scientific name	Local designation / part used	Family
01	<i>Berberis lysum</i>	Barberry; leaves	Berberidaceae
02	<i>Prosopis specigira</i>	Azghakai; branches and leaves	Fabaceae
03	<i>Citrus limon</i>	Lemon; leaves	Rutaceae
04	<i>Rosa rubiginosa</i>	Rose; pulp and leaves	Rosaceae
05	<i>Olea europaea</i>	Olive; seed	Oleaceae
06	<i>Dioscorea alata</i>	Boye; tubers and aerial parts	Dioscoreaceae
07	Eucalyptus leaves	Bargamo; leaves	Myrtaceae
08	<i>Hagenia abyssinica</i>	Kosso; flowers	Rosaceae
09	Flaxseeds	Alsi; bark	Linaceae
10	<i>Carica papaya</i>	Malakor; leaves and bark	Caricaceae
11	<i>Ocimum gratissimum</i>	Tree basil; leaves and stems	Lamiaceae
12	<i>Adenia cissampeloides</i>	Godogbo; leaves	Passifloraceae
13	<i>Limnophila aromatica</i>	Marshweed; leaves	Plantaginaceae
14	<i>Viscum album</i> (VA)	Mistletoe; leaves	Santalaceae
15	<i>Psoralea corylifolia</i> L. (PC)	Babchi; stem and leaves	Leguminosae
16	<i>Hederae folium</i>	Hydra; leaves and bark	Araliaceae
17	<i>Salvia divinorum</i>	Sage; leaves	Lamiaceae

Sample	Scientific name	Local designation / part used	Family
18	<i>Thymus serpyllum L.</i>	Thymus vulgaris; leaves	Lamiaceae
19	<i>Melissa officinalis</i>	Honey balm; bark	Lamiaceae
20	<i>Cassia tora L.</i>	Panevar; bark	Fabaceae
21	<i>Saussurea lappa</i>	Costus; leaves	Asteraceae
22	<i>Epilobium parvifolium</i>	Willowherbs; leaves	Onagraceae
23	<i>Cetraria islandica (L.) Ach.</i>	Iceland moss; leaves	Parmeliaceae

Following extraction, insoluble plant material retained by the filter cloth was discarded. The filtrate was transferred to Falcon tubes and centrifuged for 15 min. The supernatant was then filtered twice through Whatman Grade 1 qualitative filter paper using a funnel, transferred to a flask, and stored in a freezer until analysis.

Qualitative phytochemical analysis

Tannins. Tannins were screened using ferric chloride (FeCl₃). Plant extract (1.0 mL) was combined with 10 mL distilled water, mixed, and treated with 5% FeCl₃; a greenish precipitate indicated tannins. A blue or green color change after treatment of a diluted aqueous extract with 10% FeCl₃ was also taken as a positive response. For the paper-based test, aluminum foil was placed beneath the paper to prevent reagent loss, 10 µL FeCl₃ reagent was applied to two reaction zones, and the paper was dried at room temperature. Plant extract was then added, and the developed color was compared with the corresponding conventional tube test. [14]

Carbohydrates. Carbohydrates were screened using the iodine test. Iodine forms a blue to purple complex with starch. For the paper-based assay, 20 µL of plant extract was applied to the reaction zone and allowed to dry completely. Iodine was then added to the paper and the strip was left to dry for 1 day. Development of a blue or violet color was interpreted as a positive carbohydrate response.

Alkaloids. Alkaloids were screened using the procedure described in the manuscript as Dragendorff's test. Plant extract was mixed with sodium hydroxide and copper(II) sulfate solution; formation of an orange- to brownish-red precipitate was interpreted as a positive response. For the paper-based assay, 10 µL of reagent (CuSO₄, distilled water, and NaOH) was applied to chromatography paper and allowed to dry for 24 h. Subsequently, 10 µL of plant extract was added and the resulting color change was recorded.

Flavonoids. Flavonoids are phenolic secondary metabolites synthesized through the phenylpropanoid pathway and are widely reported in plants, including as part of responses to microbial stress. For screening, 10% lead acetate solution was added to 1 mL of aqueous extract. Formation of a yellow precipitate was interpreted as a positive flavonoid test.

Saponins. Saponins are widely distributed plant secondary metabolites and have been reported in many medicinal and food plants. For the test, 5 mL purified H₂O and 5 mL aqueous extract were vigorously mixed and then heated. Persistent foam formation was interpreted as a positive result. Variation among plant parts and extraction solvents was considered when interpreting the assay. Phenolic secondary metabolites arise from pathways including shikimic acid, pentose phosphate, and phenylpropanoid metabolism.

Anthraquinones. Borntrager's test was used. Three milliliters of aqueous extract was shaken with 3 mL benzene, filtered, and mixed with 5 mL of 10% ammonia. A pink, red, or violet color in the lower ammoniacal phase indicated free anthraquinones. In the second procedure, 3 mL aqueous extract was heated with 3 mL aqueous sulfuric acid and filtered while hot. The filtrate was shaken with 3 mL benzene; after phase separation, 3 mL of 10% NH₃ was added. A pink, red, or violet lower phase was taken as evidence of anthraquinone compounds.

Steroids. Two milliliters of organic extract was dissolved in 2 mL chloroform and treated with 2 mL concentrated sulfuric acid; development of a red color indicated steroids. In an additional procedure, 2 mL organic extract was dissolved in 2 mL formaldehyde and treated with acetic and sulfuric acids; development of a greenish color was interpreted as a positive steroid response.

Volatile oils. Volatile oils are common constituents of medicinal plants and have a range of traditional applications. For screening, 2 mL extract was mixed with 0.1 mL NaOH and a small amount of diluted HCl. Formation of a white precipitate was interpreted as the presence of volatile oil.

Reducing sugars. Plant extract (0.5 mL) was boiled after addition of 1 mL water and 5-8 drops of Fehling's solution. Formation of a brick-red precipitate was interpreted as a positive reducing-sugar response.

Sterols and glycosides. Liebermann's test was used as a qualitative sterol test. Two milliliters of organic extract was dissolved in 2 mL chloroform, mixed with 2 mL acetic acid, and chilled on ice before careful addition of sulfuric acid. A violet-to-blue-to-green color change indicated a steroidal nucleus or the glycone portion of a glycoside. For Salkowski's test, 2 mL of each extract was dissolved in 2 mL chloroform, followed by careful addition of 2 mL sulfuric acid and gentle mixing; a reddish-brown color indicated a steroidal ring or glycone portion of a glycoside. The Keller-Kiliani test was also used to screen acetone, methanol, and ethanol extracts for 2-deoxysugars. Two milliliters of each extract was dissolved in 2 mL glacial acetic acid containing one drop of FeCl₃ and transferred to a test tube containing 1 mL concentrated H₂SO₄. A brown ring at the interface indicated a deoxy sugar characteristic of cardenolides. [15]

Preparation of extracts for TPC and TFC analysis

TPC and TFC were treated as principal activity-related constituents. Two kilograms of fresh *L. aromatica* were cleaned and freeze-dried. The material was milled, passed through a 60-mesh sieve, and 100 g of powder was defatted with n-hexane. The solid-liquid mixture was filtered using ADVANTEC analytical filter cloth No. 2, and the retained material was dried for 6 h at 50 °C. The dehydrated, defatted *L. aromatica* (DFLA) was stored at 4 °C before extraction. DFLA (30 mg) was mixed with 1.6 mL solvent and agitated for 5 min, then centrifuged for 20 min at 13,000 × g and 25 °C. The resulting supernatant was concentrated under vacuum at 45 °C, freeze-dried for 24 h, weighed to determine extraction yield, and stored at 4 °C until analysis.

Preparation of *C. islandica* aqueous extract

For aqueous extraction of *C. islandica*, 20 g of sample was combined with 400 mL heated purified water and stirred magnetically for 15 min. The extract was filtered through Whatman No. 1 paper, frozen, and lyophilized.

Phenolic, flavonoid, and antioxidant analyses

Total phenolic content (TPC)

TPC was determined using the Folin-Ciocalteu method described by McDonald et al., with slight modifications. Freeze-dried extract was dissolved in distilled water to 50 µg/mL. Gallic acid (0-60 µg/mL) was used to construct the calibration curve. FC reagent (0.2 mL; fivefold diluted with purified H₂O) was mixed with 1.6 mL of extract or gallic-acid solution and allowed to react for 3 min. Sodium carbonate (0.2 mL, 10% w/v) was then added, and the mixture was incubated at room temperature for 30 min. Absorbance was measured at 760 nm using a Jasco V-550 UV-VIS spectrophotometer (Tokyo, Japan). TPC was expressed as milligrams gallic acid equivalents per gram of defatted *L. aromatica* (mg GAE/g DFLA). [16,17]

Total flavonoid content (TFC)

TFC was determined using the aluminum chloride colorimetric method, with minor modifications. Extracts were diluted in methanol to 100 µg/mL. Quercetin (0-100 µg/mL) in methanol was used to construct the calibration curve. Diluted extract or quercetin solution (2.0 mL) was combined with 0.1 mL of 10% (w/v) aluminum chloride solution and 0.1 mL of 0.1 mM potassium acetate solution. [17,18]

The mixtures were incubated for 30 min at room temperature, after which absorbance was measured at 415 nm using a UV-VIS spectrophotometer. TFC was expressed as milligrams quercetin equivalents per gram of defatted *L. aromatica* (mg QCE/g DFLA).

Steam-distillation procedure

For the condensation/steam-distillation procedure, 50 g of each dried plant material was placed in a round-bottom flask with 200 mL H₂O and heated. Volatile components were condensed and collected in receiving flasks over a 2-h distillation period. Oils accumulating above the aqueous phase were separated using a separating funnel and stored in bijoux bottles under refrigeration. The recovered oil mass was recorded and used to calculate percentage yield. [19]

Solvent extraction and fractionation

Solvent extraction was performed using the Soxhlet approach described in the cited natural-product extraction procedures. Forty grams of dried foliage was wrapped in filter paper and placed in the extraction chamber. A round-bottom flask containing 250 mL absolute ethanol was connected below the extractor and reflux apparatus above. Petroleum ether was used instead of ethanol for extraction of *C. citratus* oil, as described in the source method. Extraction was performed for 8 h with approximately 18 reflux cycles. Solvent was recovered after sample removal, and the recovered oils were transferred to bijoux bottles and stored in a freezer. Percentage yield was calculated from the recovered material. For fractionation, oil was placed in a clean separating funnel and mixed with 10 mL of 50% aqueous ethanol followed by 40 mL additional aqueous ethanol and 50 mL of an ether/chloroform solvent mixture. After vigorous shaking, the mixture was allowed to stand for approximately 1 h for phase separation. The aqueous lower phase and organic upper phase were collected separately and dried by evaporation. [19]

Ferric reducing antioxidant power (FRAP) analysis

The reducing/antioxidant activity of medicinal-plant extracts was assessed using the FRAP-related ferric reduction procedure. [11]

A phosphate buffer (0.2 M) was prepared using 8 g sodium chloride, 1.44 g disodium hydrogen phosphate, 0.2 g potassium chloride, and 0.24 g potassium dihydrogen phosphate in 800 mL water; pH was adjusted to 6.6 and the final volume brought to 1,000 mL with deionized water. Stock solutions included 1% potassium ferricyanide (1 g/100 mL dH₂O), 10% trichloroacetic acid (10 g/100 mL dH₂O), 0.1% ferric chloride (100 mg/100 mL dH₂O), and 0.1% ascorbic acid (1 mg/mL H₂O). The assay is based on reduction of ferric species in the presence of antioxidants, with absorbance of the resulting colored mixture measured at 700 nm.

The percentage response was calculated using the following equation:

$$\text{FRAP \% restriction} = ((\text{AB} - \text{AA}) / \text{AB}) \times 100$$

ABTS radical-scavenging analysis

ABTS radical-scavenging activity was evaluated using the ABTS radical-cation decolorization approach. A 7 mM ABTS solution and 2.45 mM potassium persulfate were mixed at a 1:1 ratio and stored in the dark at room temperature for 12-16 h to generate ABTS⁺ radical cations. The working ABTS⁺ solution was diluted with methanol to an absorbance of 0.700 at 734 nm. Diluted ABTS⁺ solution (3.995 mL) was mixed with 5 μ L plant extract, incubated in the dark for 30 min, and measured spectrophotometrically. Solvent blanks were included. Ethanol, methanol, and aqueous plant extracts were mixed thoroughly; approximately 25 μ L of each sample was added to the working solution and vortexed. For the tube-format procedure, 10 μ L sample or Trolox control was added to the designated tubes and 10 μ L assay buffer was used for negative controls, followed by approximately 20 μ L analyte or Trolox and 100 μ L ABTS solution. [12]

Reactions were allowed to proceed for approximately 5 min before addition of 50 μ L stopping solution. Trolox was used as the standard, and absorbance was measured at 734 nm.

The percentage response was calculated using the following equation:

$$\text{ABTS}^+ \% \text{ restriction} = ((\text{AB} - \text{AA}) / \text{AB}) \times 100$$

Antioxidant analysis of *C. islandica*

Thiocyanate method

Antioxidant activity of *C. islandica* was evaluated using the thiocyanate method, an approach used in studies of antioxidant effects on lipid oxidation and oxidative stress. *C. islandica* (10 mg) was dissolved in 10 mL water. Linoleic acid was combined with potassium phosphate buffer (2.5 mL, 0.04 M, pH 7.0), and 1 mg *C. islandica* in 1 mL water was added. The mixture was incubated at 37 °C. Peroxide formation was monitored by measuring absorbance at 500 nm after reaction with FeCl₂ and thiocyanate at different incubation times. Solutions without added extract served as controls. [20]

DPPH assay

The DPPH method was used to assess the free-radical-scavenging capacity of *C. islandica* using the conventional Blois approach and related antioxidant methods. Three milliliters of *C. islandica* aqueous solution at different doses (50-250 μ g) was added to a 0.1 mM DPPH solution in ethanol. Absorbance was measured at 517 nm after 30 min. Lower absorbance indicated greater radical-scavenging activity. DPPH concentration in the reaction medium was quantified using the calibration relationship below. [13]

DPPH concentration in the reaction medium was calculated as:

$$\text{Absorbance} = 2.492 \times [\text{DPPH}] + 0.039$$

Statistical analysis

Each assessment was performed in at least duplicate, and mean values with the reported dispersion measures were presented. Data were analyzed using Minitab software. One-way ANOVA was used for statistical comparisons, with $p < 0.05$ considered statistically significant. Correlation coefficients (R) were calculated to assess the relationship between TPC and TFC.

RESULTS

Tannin detection

Tannin results obtained by the conventional procedure and the paper-based colorimetric strips are summarized in Table 2 and Figure 2. *Berberis lysum* and *Prosopis specigira* were negative by both methods, whereas *Citrus limon*, *Rosa rubiginosa*, and

Olea europaea were positive by both methods. Thus, for the samples tested, the paper-based strips produced the same qualitative tannin classifications as the conventional procedure.

Table 2. Tannin detection by the standard procedure and paper-based strips.

Sample	Standard procedure	Paper strips
<i>Berberis lysum</i>	Negative	Negative
<i>Prosopis specigira</i>	Negative	Negative
<i>Citrus limon</i>	Positive	Positive
<i>Rosa rubiginosa</i>	Positive	Positive
<i>Olea europaea</i>	Positive	Positive

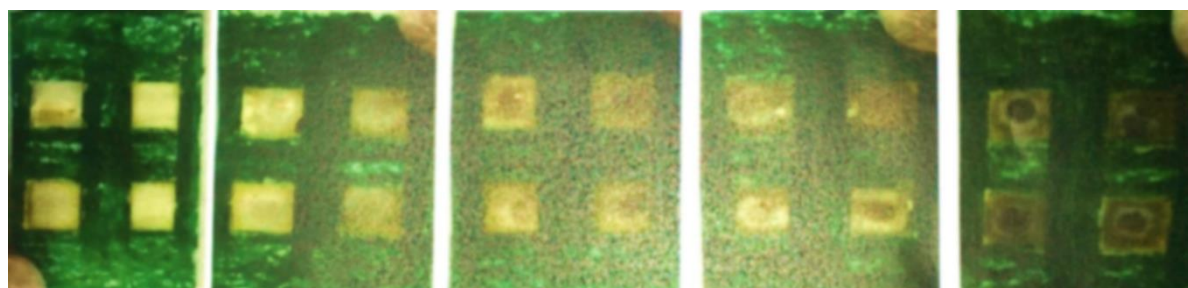


Figure 2. Tannin identification using paper-based colorimetric strips.

Carbohydrate detection

Carbohydrate screening results are presented in Table 3 and Figure 3. *Berberis lysum*, *Prosopis specigira*, *Citrus limon*, *Rosa rubiginosa*, and *Olea europaea* were negative for carbohydrates by both the conventional procedure and the paper-based strip method. No visible color response indicative of carbohydrates was observed for these samples.

Table 3. Carbohydrate detection by the standard procedure and paper-based strips.

Sample	Standard procedure	Paper strips
<i>Berberis lysum</i>	Negative	Negative
<i>Prosopis specigira</i>	Negative	Negative
<i>Citrus limon</i>	Negative	Negative
<i>Rosa rubiginosa</i>	Negative	Negative
<i>Olea europaea</i>	Negative	Negative

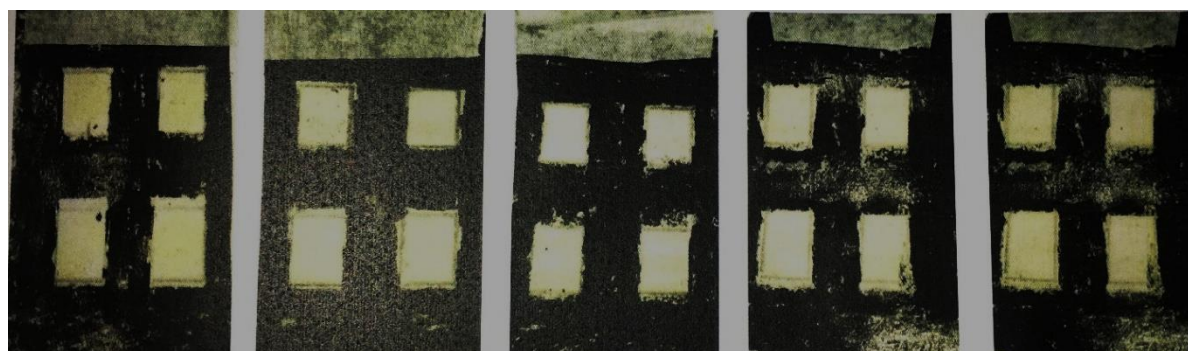


Figure 3. Carbohydrate identification using paper-based colorimetric strips.

Alkaloid detection

Alkaloid results are summarized in Table 4 and Figure 4. *Berberis lysum*, *Citrus limon*, *Rosa rubiginosa*, and *Olea europaea* were positive by both methods, whereas *Prosopis specigira* was negative by both methods. The paper-based strip method therefore reproduced the qualitative classifications obtained with the conventional procedure for all five samples evaluated.

Table 4. Alkaloid detection by the standard procedure and paper-based strips.

Sample	Standard procedure	Paper strips
<i>Berberis lysum</i>	Positive	Positive
<i>Prosopis specigira</i>	Negative	Negative
<i>Citrus limon</i>	Positive	Positive
<i>Rosa rubiginosa</i>	Positive	Positive
<i>Olea europaea</i>	Positive	Positive



Figure 4. Alkaloid identification using paper-based colorimetric strips.

Flavonoid detection

Flavonoid screening results are shown in Table 5. *Carica papaya*, *Ocimum gratissimum*, and *Adenia cissampeloides* were positive by both the conventional procedure and the paper-based strip method. The qualitative classifications were identical between the two approaches for the three samples evaluated.

Table 5. Flavonoid detection by the standard procedure and paper-based strips.

Sample	Standard procedure	Paper strips
<i>C. papaya</i>	Positive	Positive
<i>O. gratissimum</i>	Positive	Positive
<i>A. cissampeloides</i>	Positive	Positive

Saponin detection

Saponin screening results are shown in Table 6. *Carica papaya* and *Adenia cissampeloides* were positive, whereas *Ocimum gratissimum* was negative, by both the conventional procedure and the paper-based strip method. The strip responses therefore agreed with the conventional results for all samples tested.

Table 6. Saponin detection by the standard procedure and paper-based strips.

Sample	Standard procedure	Paper strips
<i>C. papaya</i>	Positive	Positive
<i>O. gratissimum</i>	Negative	Negative
<i>A. cissampeloides</i>	Positive	Positive

Anthraquinone, steroid, and glycoside detection

Anthraquinone screening results are summarized in Table 7. The conventional procedure and paper-based strip method both produced negative anthraquinone responses for the tested material.

Steroids

Steroid results are also summarized in Table 7. *Carica papaya*, *Ocimum gratissimum*, and *Adenia cissampeloides* produced positive steroid responses using the conventional procedure, and the paper-based strips yielded the same qualitative result.

Glycosides

Glycoside results are summarized in Table 7. *Carica papaya*, *Ocimum gratissimum*, and *Adenia cissampeloides* were positive using the conventional procedure, and the paper-based strips yielded the same qualitative classification.

Table 7. Detection of anthraquinones, steroids, and glycosides by the standard procedure and paper-based strips.

Phytochemical	Standard procedure	Paper strips
Anthraquinones	Negative	Negative
Steroids	Positive	Positive
Glycosides	Positive	Positive

Reducing-sugar and volatile-oil screening

Qualitative results for *Viscum album*, *Psoralea corylifolia* L., and *Artemisia herba* are summarized in Table 8. *Viscum album* was negative, whereas *Psoralea corylifolia* L. and *Artemisia herba* were positive, and the paper-based strips reproduced the corresponding conventional classifications.

Table 8. Qualitative screening results for *V. album*, *P. corylifolia* L., and *A. herba* by the standard procedure and paper-based strips.

Sample	Standard procedure	Paper strips
<i>Viscum album</i>	Negative	Negative
<i>Psoralea corylifolia</i> L.	Positive	Positive
<i>Artemisia herba</i>	Positive	Positive

Extraction yield, total phenolic content, and total flavonoid content

Extraction yield, TPC, and TFC values for freeze-dried *L. aromatica* are presented in Table 9. Extraction yield was highest with 100% methanol ($26.06 \pm 1.64\%$), followed by water ($25.58 \pm 1.04\%$), 100% ethanol ($17.03 \pm 2.66\%$), and 100% acetone ($12.33 \pm 1.53\%$). The higher yields obtained with methanol and water may reflect recovery of nonphenolic constituents in addition to phenolics, because proteins and carbohydrates can show greater solubility in methanol and water than in ethanol or acetone. TPC values were calculated from the calibration curve $y = 50x + 7.75$ ($R^2 = 0.9894$) and expressed as mg GAE/g DFLA. TPC was 40.50 ± 0.88 mg GAE/g for 100% ethanol, 40.30 ± 0.20 for 100% acetone, 31.50 ± 1.60 for 100% methanol, and 6.25 ± 0.24 for water. The water extract had a substantially lower TPC than the organic-solvent extracts ($p < 0.01$), whereas the TPC of the ethanol extract was not considerably higher than that of the acetone extract. [21,22]

Table 9. Extraction yield, TPC, and TFC of freeze-dried *L. aromatica*.

No.	Solvent system	Extraction yield (%)	TPC (mg GAE/g)	TFC (mg QCE/g)
01	H ₂ O	25.58 ± 1.04	6.25 ± 0.24	4.04 ± 0.08
02	Methanol (100%)	26.06 ± 1.64	31.50 ± 1.60	15.42 ± 0.40
03	Ethanol (100%)	17.03 ± 2.66	40.50 ± 0.88	31.11 ± 0.43
04	Acetone (100%)	12.33 ± 1.53	40.30 ± 0.20	30.86 ± 0.14

GAE, gallic acid equivalent; QCE, quercetin equivalent; TFC, total flavonoid content; TPC, total phenolic content. Solvent system is expressed relative to H₂O; extraction yield is expressed as $100 \times (\text{g dry extract} / \text{g dry plant material})$.

TFC showed a pattern similar to TPC. The highest TFC was obtained with 100% ethanol (31.11 ± 0.43 mg QCE/g), followed by 100% acetone (30.86 ± 0.14), 100% methanol (15.42 ± 0.40), and water (4.04 ± 0.08). TFC decreased as the water content of the ethanol- or acetone-based extraction system increased. TPC and TFC were strongly correlated ($R = 0.923$), suggesting that flavonoids represented a major component of the phenolic fraction in *L. aromatica*. This pattern was reported to be comparable with phenolic profiles described for guava and Pisang Mas banana. [22]

Table 10. Oil extraction yields for *C. papaya*, *A. cissampeloides*, and *O. gratissimum*.

No.	Procedure	<i>C. papaya</i> (%)	<i>A. cissampeloides</i> (%)	<i>O. gratissimum</i> (%)
01	Wet purification	5.60	5.44	5.80
02	Solvent isolation	7.40	6.75	6.30

The quantitative oil-yield results are summarized in Table 10. Steam distillation produced the highest yield for *O. gratissimum* (5.80%), whereas solvent extraction produced the highest yield for *C. papaya* (7.40%).

FRAP analysis

Table 11. FRAP responses of medicinal-plant extracts measured at 700 nm.

No.	Plant	EE	ME	AE
01	<i>Hederae folium</i>	48.70 ± 0.66	47.13 ± 0.46	14.84 ± 1.28
02	<i>Salvia divinorum</i>	48.52 ± 1.80	46.96 ± 0.16	11.02 ± 0.38
03	<i>Thymus serpyllum L.</i>	48.35 ± 0.43	44.15 ± 0.46	14.52 ± 0.60
04	<i>Melissa officinalis</i>	48.12 ± 1.65	47.38 ± 1.74	14.94 ± 1.76
05	<i>Cassia tora L.</i>	47.84 ± 0.77	45.26 ± 0.94	18.85 ± 0.48
06	<i>Saussurea lappa</i>	47.78 ± 1.63	47.00 ± 0.78	12.55 ± 1.18

Values are reported as mean $\pm$ SD ($\mu\text{g/mL}$) for free-radical-scavenging/reducing activity.

Reducing capacity was evaluated through Fe^{3+} to Fe^{2+} reduction. The assay is based on electron donation by antioxidants, including phenolic acids and flavonoids, which reduces the ferric-ferricyanide complex and produces a chromogenic ferrous species. [23]

Absorbance of the resulting blue-green reaction product was measured at 700 nm. The medicinal-plant extracts showed different ferric-reducing capacities, with detailed EE, ME, and AE values presented in Table 11. At 500 $\mu\text{g/mL}$, L-ascorbic acid showed $85.48 \pm 1.56\%$ activity. Across the listed plant extracts, the overall reducing-power sequence was reported as L-ascorbic acid > EE > ME > AE. The reducing behavior was attributed to reductone-type constituents capable of donating hydrogen atoms or reacting with peroxide species. Further work is needed to isolate and identify individual phenolic components with potential therapeutic relevance. [23,24]

ABTS radical-scavenging analysis

ABTS+ radical-scavenging activity was evaluated for plant extracts prepared in different solvents and compared with Trolox and standard ascorbic acid. The ABTS radical cation was generated by reaction of ABTS with potassium persulfate, and absorbance was measured at 734 nm after a 30-min dark incubation. Detailed results are presented in Table 12.

Table 12. ABTS radical-scavenging responses of medicinal-plant extracts measured at 734 nm.

No.	Plant	EE	ME	AE
01	<i>Hederae folium</i>	51.42 ± 1.54	49.38 ± 1.42	15.04 ± 1.90
02	<i>Salvia divinorum</i>	51.16 ± 1.65	50.96 ± 1.56	13.35 ± 0.16
03	<i>Thymus serpyllum L.</i>	50.74 ± 1.22	50.64 ± 0.63	15.42 ± 0.76
04	<i>Melissa officinalis</i>	50.52 ± 1.58	47.46 ± 0.96	16.55 ± 1.24
05	<i>Cassia tora L.</i>	50.25 ± 1.32	49.47 ± 1.62	19.60 ± 1.46
06	<i>Saussurea lappa</i>	48.96 ± 0.56	46.44 ± 0.38	15.26 ± 0.69

Values are reported as mean $\pm$ SD ($\mu\text{g/mL}$) for free-radical-scavenging activity.

Standard ascorbic acid showed the highest reported ABTS radical-scavenging activity ($88.64 \pm 1.22\%$). The EE, ME, and AE values for *Hederae folium*, *Salvia divinorum*, *Thymus serpyllum L.*, *Melissa officinalis*, *Cassia tora L.*, and *Saussurea lappa* are summarized in Table 12. Overall, the ethanol extracts showed the greatest radical-scavenging capacity, followed by methanol and aqueous extracts. The assay can be applied to both aqueous and lipophilic antioxidant fractions. The observed activity is consistent with previous reports describing antioxidant and related biological properties of phenolic- and flavonoid-containing plant extracts. [10,25]

Antioxidant activity of *C. islandica*

C. islandica showed strong antioxidant activity across the concentrations evaluated. Using the thiocyanate method, aqueous *C. islandica* extract at 50-500 μg inhibited lipid peroxidation in the linoleic-acid emulsion by 96%, 99%, 100%, and complete inhibition, respectively, compared with 77% inhibition for 500 μg α -tocopherol (Figure 5). The percentage inhibition was calculated using the equation below.

$$\% \text{ inhibition} = ((A_0 - A_1) / A_0) \times 100$$

where A_0 represents the absorbance of the control reaction and A_1 represents the absorbance term defined for the *C. islandica* aqueous-extract assay.

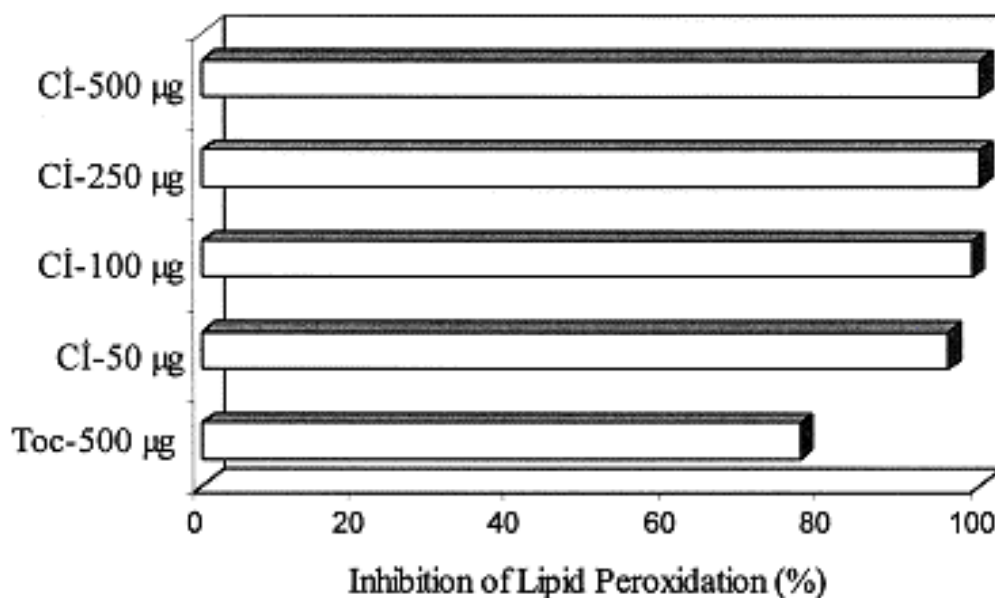


Figure 5. Inhibition of lipid peroxidation by aqueous *C. islandica* extract at different doses compared with tocopherol in a linoleic-acid emulsion. Toc, tocopherol.

DPPH analysis of *C. islandica*

The aqueous extract of *C. islandica* significantly reduced DPPH radical concentration ($P < 0.05$) relative to the control (Figure 6). Quercetin and BHA were used as reference standards. Results were based on five parallel measurements and are reported as mean $\pm$ SD. $P < 0.05$ was considered significant and $P < 0.01$ highly significant.

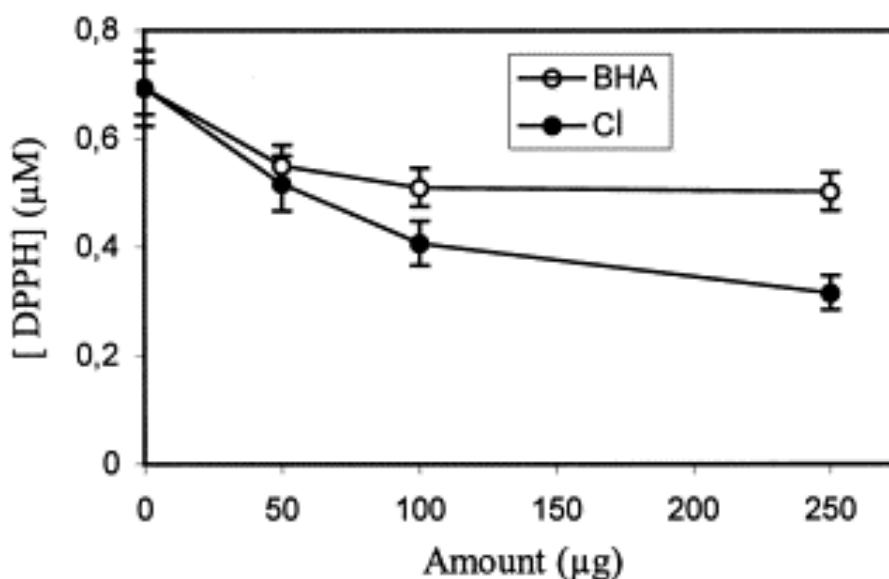


Figure 6. DPPH radical-scavenging activity of aqueous *C. islandica* extract compared with BHA and quercetin. Results are mean $\pm$ SD of five parallel measurements; $P < 0.01$ versus control. BHA, butylated hydroxyanisole.

DISCUSSION

The main finding of this study is the consistent qualitative agreement between the paper-based strip and the corresponding conventional procedures for the samples evaluated. Tannin, carbohydrate, alkaloid, flavonoid, saponin, anthraquinone, steroid, glycoside, reducing-sugar, and volatile-oil screening produced the same positive or negative classifications when the strip result was compared with the standard test. This agreement is important because the strip was not based on a new reaction chemistry; instead, the established color or precipitation response was transferred to a small paper reaction zone. The practical contribution is therefore the format. By confining the reaction within wax-defined 1×1 cm areas, several reactions can be arranged on a compact support and visually compared without the volume of glassware required for parallel tube testing. The observed room-temperature stability over the two-month monitoring period also supports the feasibility of storing prepared strips for short-term use, although the present work did not quantify reagent degradation or establish a formal shelf-life specification. The findings should therefore be interpreted as evidence of qualitative feasibility rather than as a completed validation of analytical stability.

Paper-based colorimetric analysis is attractive because cellulose is inexpensive, portable, compatible with microliter-scale reagents, and easily patterned into multiple test zones. The present design is deliberately simpler than many microfluidic paper-based analytical devices, but it shares the same general advantages of low material consumption and direct visual readout [7,8]. The results show that this simplicity can be useful for preliminary phytochemical screening, particularly where the analytical question is whether a conventional reaction is visibly positive or negative. At the same time, visual interpretation is a central limitation. Ambient lighting, camera exposure, paper background, reagent distribution, extract color, operator perception, and drying time can all influence the apparent color. The study did not establish limits of detection, diagnostic sensitivity or specificity, inter-reader agreement, or a quantitative color threshold. These issues should be addressed in future work through standardized illumination, reference color charts, replicate-reader assessment, and smartphone-based image analysis. Such improvements would preserve the low-cost format while reducing subjectivity.

The quantitative measurements provide a complementary view of the plant extracts and should not be interpreted as validation metrics for the strip itself. In *L. aromatica*, extraction yield was highest with 100% methanol, whereas the highest TPC and TFC values were obtained with 100% ethanol and were closely followed by 100% acetone. The strong positive correlation between TPC and TFC ($R = 0.923$) indicates that flavonoids constituted an important component of the phenolic fraction measured in these extracts. Solvent-dependent differences of this type are expected because extraction efficiency reflects both solvent polarity and the chemical diversity of the matrix [9,21,22]. The FRAP and ABTS data also showed clear differences among ethanol, methanol, and aqueous extracts. These assays probe related but non-identical antioxidant mechanisms, and comparison across assays is therefore most informative when the measured responses are considered together rather than treated as interchangeable [10-12]. The strong activity observed for aqueous *C. islandica* in the thiocyanate and DPPH experiments further supports the presence of compounds capable of inhibiting lipid oxidation or scavenging radicals under the conditions used [13,20]. These observations are consistent with the broader literature linking phenolic-rich plant extracts with reducing and radical-scavenging activity [23-25].

Several limitations define the scope of the conclusions. First, the strip was evaluated as a qualitative screen on selected plant samples, so agreement with the conventional tests cannot be generalized to all plant matrices or concentration ranges. Second, some extracts are naturally colored, which can complicate visual interpretation and may mask weak reagent responses. Third, the study used a simple wax barrier and manual reagent deposition; manufacturing reproducibility, batch-to-batch variability, humidity sensitivity, and long-term reagent stability were not systematically quantified. Fourth, the additional antioxidant and phenolic analyses were performed on selected extracts and should be viewed as chemical characterization rather than evidence that the paper strip quantitatively predicts antioxidant capacity. Finally, the current format relies on chemical reagents that may require careful handling and disposal. Future development should therefore focus on larger validation sets, standardized reagent loading, controlled storage studies, objective image-based color measurement, and definition of analytical performance characteristics. With those refinements, the platform could become a useful field or teaching tool for rapid preliminary phytochemical screening, while confirmatory or quantitative analyses would continue to require appropriate laboratory methods. The current findings also do not establish clinical diagnostic performance, pharmacological efficacy, or safety of the tested plant materials. Those questions require separate study designs, appropriate reference standards, and validation in the intended application setting. Maintaining this distinction is important because a positive phytochemical reaction indicates the presence of a responsive chemical class under the assay conditions, not a therapeutic effect.

CONCLUSION

This study developed a wax-patterned paper-based colorimetric strip and compared its qualitative phytochemical classifications with conventional procedures. For the tested samples, the strip reproduced the corresponding positive or negative results for the phytochemical classes evaluated. The accompanying extraction, TPC, TFC, FRAP, ABTS, thiocyanate, and DPPH analyses demonstrated solvent-dependent differences in the selected medicinal-plant extracts without altering the qualitative purpose of the paper device. The strip therefore represents a simple and low-cost screening format rather than a fully validated quantitative sensor. Further work should standardize reagent deposition, storage conditions, color interpretation, and analytical performance across larger and more diverse sample sets before routine analytical use.

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