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Comparative evaluation of different extraction techniques on phytochemicals and antioxidant activity of selected medicinal plants

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Abstract
Medicinal plants are valuable sources of phytochemicals with significant pharmacological properties, including antioxidant, antimicrobial and anti-inflammatory effects. Efficient extraction methods are crucial to maximize the therapeutic potential of these bioactive compounds. In this study, we comparatively examined the phytochemical profiles and antioxidant activities of three ethnomedicinally important plants *Clitoria ternatea*, *Cassia tora* and *Sida acuta*, using three conventional extraction techniques: maceration, decoction and Soxhlet extraction. These species are widely used by rural communities in the Balrampur district of Chhattisgarh, yet limited research exists regarding the most suitable extraction method for optimal bioactivity. Qualitative screening confirmed the presence of major phytochemical classes in all extracts. Across all three plants *Clitoria ternatea*, *Cassia tora*, and *Sida acuta,* the soxhlet extraction (CT-SOX, CA-SOX, SA-SOX) generally yielded more intense reactions/presence (++ to +++) for a broad range of phytochemicals compared to Maceration (MAC) and Decoction (DEC), indicating its superior efficiency in extracting bioactive compounds. DPPH assay showed the strongest antioxidant activity in CT-SOX and SA-SOX extracts i.e. 78.5±4.5% and 73.3±3.8% inhibition, respectively. Decoction generally showed lower bioactive recovery, especially in Cassia tora and Sida acuta, while maceration offered moderate antioxidant performance. Significant differences (p<0.05) were observed in qualitative and antioxidant activity among extraction methods and species. Our study established soxhlet as the most effective method for recovering antioxidants. The findings are valuable for standardizing herbal formulations and enhancing the evidence-based application of these plants in traditional and modern healthcare systems.

Keywords: Medicinal plants, phytochemicals, antioxidant activity, *Clitoria ternatea*, soxhlet extraction

1. Introduction
Medicinal plants have played a pivotal role in traditional healthcare systems across the world and are now increasingly validated by modern pharmacology for their therapeutic applications. These plants are rich in diverse phytoconstituents such as phenolics, flavonoids, alkaloids, glycosides, tannins and steroids, which contribute to various bioactivities, particularly antioxidant, anti-inflammatory, antimicrobial and antidiabetic effects (Roy *et al*., 2022; Riaz *et al*., 2023; Mwangi *et al*., 2024) [16, 15, 11]. With rising interest in plant-based alternatives to synthetic drugs, efficient extraction and characterization of bioactive constituents are essential for maximizing their medicinal utility (Siddiqui *et al*., 2023) [20]. Extraction is a critical step in isolating phytochemicals from plant matrices as the pharmacological potential of medicinal plants is largely determined by the efficiency and appropriateness of the extraction method employed (Jha and Sit, 2022) [7]. Common extraction techniques like maceration, decoction and Soxhlet extraction vary in terms of solvent penetration, thermal exposure, extraction time and yield. Maceration is a simple, low-cost technique suitable for heat-sensitive compounds, but often provides low extraction efficiency due to limited solvent percolation (Cao *et al*., 2025) [4]. Decoction, typically used in traditional medicine systems, is effective for water-soluble bioactives but unsuitable for thermolabile phytochemicals due to high temperatures (Kokilananthan *et al*., 2022) [8]. In contrast, Soxhlet extraction enables exhaustive recovery of compounds through repeated solvent reflux, often yielding higher phenolic and flavonoid contents, although prolonged heat exposure may lead to degradation of sensitive molecules (Kokilananthan *et al*., 2022; Cao *et al*., 2025) [8, 4].

Several comparative studies have attempted to assess the efficiency of these methods. For instance, *Ocimum sanctum* and *Terminalia chebula* were found to yield higher antioxidant activity when extracted *via* Soxhlet compared to decoction or maceration (Chaudhary *et al*., 2020; Kumar *et al*., 2021) [5, 9]. Similarly, *Azadirachta indica* demonstrated greater total phenolic content in ethanolic Soxhlet extracts than aqueous decoctions (Veerendrakumar *et al*., 2023) [22]. However, many of these studies focus either on a single plant species or a single extraction method, lacking a comprehensive cross-method and cross-species evaluation under uniform experimental conditions. This is especially true for lesser-studied yet ethnomedicinally important plants such as *Clitoria ternatea*, *Cassia tora* and *Sida acuta*. These three species are traditionally used in various rural and tribal communities of India. *Clitoria ternatea* (commonly known as butterfly pea) is known for its cognitive-enhancing, antistress and anxiolytic properties (Multisona *et al*., 2023) [10]. *Cassia tora* is traditionally used for skin diseases, constipation and inflammation due to its rich anthraquinone and flavonoid content (Bhandirge *et al*., 2016) [2]. *Sida acuta*, widely used in Ayurvedic medicine, is valued for its antipyretic, wound healing and hepatoprotective potential (Ogunmoyole *et al*., 2022; Riaz *et al*., 2023) [13, 15]. These plants are abundantly available and frequently used by the indigenous population of the studied region, yet systematic studies exploring the best-suited extraction methods for maximizing their phytochemical and antioxidant potential are scarce.

This lack of comparative extraction studies has created a critical research gap. Although individual reports exist, there is no consolidated research comparing these three plants across the three major extraction techniques under identical conditions. Moreover, most previous studies do not correlate extraction efficiency with biological (antioxidant) performance, which is crucial for phytopharmaceutical development. In view of this, the present study was undertaken to comparatively examine the phytochemical content and antioxidant potential of *Clitoria ternatea*, *Cassia tora* and *Sida acuta* using maceration, decoction and soxhlet extraction methods. By integrating qualitative, quantitative and biological evaluation of the extracts, this work aims to establish the most effective extraction approach for each plant and fill the knowledge gap regarding method-specific bioactive recovery. This is especially relevant for promoting evidence-based use of these ethnobotanically important species in local traditional medicine systems and validating them for future therapeutic development.

2. Materials and Methods

2.1 Site selection

The study was conducted using plant samples collected from forest area of Wadrafnagar Block of Balrampur district (Chhattisgarh) India, known for its diverse populations of medicinal flora. The sites were chosen based on accessibility, natural habitat suitability and availability of target medicinal plants: *Clitoria ternatea*, *Cassia tora* and *Sida acuta*.

2.2 Plant sample collection

Fresh and healthy flowers of *Clitoria ternatea* and leaves of *Cassia tora* and *Sida acuta* were collected. After collection, flowers of *Clitoria ternatea* (CT) and leaves of *Cassia tora* (CA) and *Sida acuta* (SA) were plucked from plants and rinsed with tap water followed by distilled water to remove dust and debris. All samples were shade-dried at room temperature (25-27 °C) for 7-10 days, then pulverized using an electric grinder and the powder was stored in airtight containers for further analysis.

2.3 Preparation of plant extract

Extract of collected plant samples were prepared by maceration, decoction and soxhlet extraction methods. The extracts were labeled based on plant species and extraction method as *Clitoria ternatea* extracts as CT-MAC, CT-DEC and CT-SOX; *Cassia tora* extracts as CA-MAC, CA-DEC and CA-SOX and *Sida acuta* extracts as SA-MAC, SA-DEC and SA-SOX, representing maceration, decoction and soxhlet extraction methods respectively.

a) Maceration

10 g of leaf powder was soaked in 100 ml of 70% ethanol in a conical flask, sealed and kept at room temperature (~25-30°C) for 72 hours with occasional shaking. The extract was filtered (Whatman No.1) and concentrated using a rotary evaporator under reduced pressure.

b) Decoction

10 g of plant powder was boiled in 100 ml of distilled water for 30 minutes. After cooling, the mixture was filtered and the extract was concentrated by evaporation on a water bath at 50°C.

c) Soxhlet Extraction

10 g of plant powder was packed into a thimble and extracted using 90% ethanol in a Soxhlet apparatus for 6-8 hours. The solvent was removed by rotary evaporation and the concentrated extract was stored at 4 °C in amber bottles for further use.

2.4 Qualitative phytochemical analysis

The phytochemical analysis of macerated, decocted and soxhlet extracts of *Clitoria ternatea* (CT) and leaves of *Cassia tora* (CA) and *Sida acuta* (SA) were carried out using standard protocols to determine the presence of secondary metabolites. The plant extracts were screened for the presence of various secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, glycosides, steroids, terpenoids, saponins and anthocyanins by following protocols given in Harborne *et al*., (1998) [6]. The results were recorded based on the appearance and intensity of characteristic color changes or precipitate formation.

The intensity of qualitative phytochemical assay solutions of plant extracts was measured using a UV-Vis spectrophotometer. For each test (as described in Section 2.4), the color developed was measured at the wavelength corresponding to the peak absorbance (λmax) of the respective chromophore, typically ranging between 400-700 nm depending on the test. The absorbance of each sample was measured against a reagent blank (containing only the solvent and reagent), with all solutions prepared under identical conditions including reagent volume, reaction time, and temperature to ensure consistency. The intensity of phytochemical presence was then graded based on the absorbance values as follows: (-) for no or negligible absorbance (<0.1), (+) for low absorbance (0.1-0.3), (++) for moderate absorbance (0.31-0.6) and (+++) for high absorbance (>0.6). All tests were performed in triplicate to ensure reliability and reproducibility.

2.5 Antioxidative capacity

Antioxidant activity of each extract was assessed by following Brand-Williams *et al*., (1995) [3]. For this DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed. 1 ml of extract (1 mg/ml) was mixed with 1 ml of 0.1 mM DPPH in methanol. The solution was incubated in the dark for 30 minutes at room temperature. Absorbance was read at 517 nm using a UV-Vis spectrophotometer. DPPH solution without sample was used as control sample for each sample. Antioxidant activity (%) was calculated using the formula:

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where,

$A_{control}$ = Absorbance of the control (DPPH solution without sample)

A_{sample} = Absorbance of the sample (DPPH solution with plant extract)

2.6 Statistical analysis

All experiments were conducted at least twice, with five independent replicates for each sample. All the results are presented as mean values ± standard deviation. Relationships among the parameters were analyzed using Pearson's correlation coefficient. One-way Analysis of Variance (ANOVA) was employed to assess significant differences among means, followed by Duncan's Multiple Range Test (DMRT) for post hoc comparison.

3. Results

3.1 Qualitative phytochemical analysis

The qualitative phytochemical screening of plant extracts revealed notable differences in the presence and intensity of secondary metabolites depending on both the plant species and the extraction method. Across all three plants i.e. *Clitoria ternatea*, *Cassia tora*, and *Sida acuta,* the soxhlet extraction (CT-SOX, CA-SOX, SA-SOX) generally yielded more intense reactions/presence (++ to +++) for a broad range of phytochemicals compared to Maceration (MAC) and Decoction (DEC), indicating its superior efficiency in extracting bioactive compounds. In *Clitoria ternatea*, the soxhlet extract (CT-SOX) showed strong presence (+++) of alkaloids, flavonoids, phenolics and tannins, while moderate (++), or weak (+) presence were observed in CT-MAC and CT-DEC. Similarly, soxhlet extract of *Cassia tora* (CA-SOX) exhibited high intensity (+++) for alkaloid, flavonoids and phenolics, whereas the decoction extract (CA-DEC) showed comparatively lower intensity and complete absence (-) was observed for saponin and anthocyanins. In the case of *Sida acuta*, SA-SOX presented strong (+++) reactions for alkaloids, flavonoids and phenolics whereas SA-MAC and SA-DEC demonstrated moderate (++) or weak (+) intensities across most phytochemicals. This pattern indicates a statistically relevant trend, where soxhlet extracts across all species presented significantly higher qualitative presence of phytochemicals (χ^2 test, $p<0.05$), supporting soxhlet extraction as a more efficient technique for secondary metabolite recovery.

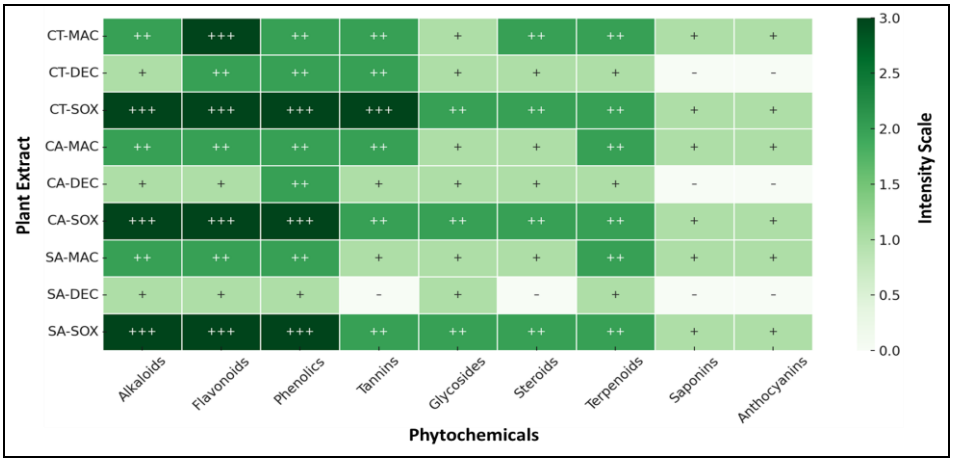

Fig 1: Heatmap showing phytochemical presence intensity by sample and extraction method. Darker green indicates higher intensity and white indicates absence of phytochemicals (+++ = Strong presence, ++ = Moderate presence, + = Weak presence, - = Absent)

3.2 Antioxidant activity (DPPH radical scavenging assay)

The antioxidant activity as measured by DPPH radical scavenging assay also followed a similar trend. The soxhlet extracts of all three plants (CT-SOX, CA-SOX, SA-SOX) exhibited the highest percentage inhibition (>70%) followed by Maceration extracts (MAC) which showed moderate activity (50-70%). Decoction extracts displayed the lowest antioxidant activity, often falling below 50%, with CA-DEC and SA-DEC exhibiting values <40%, indicating poor free radical scavenging ability. The statistical analysis confirmed that soxhlet extracts had significantly higher antioxidant potential compared to both maceration and decoction ($p<0.05$). Thus, the DPPH assay results corroborate the phytochemical screening observations, establishing a clear correlation between metabolite abundance and antioxidant efficacy, particularly favoring Soxhlet extraction as the most potent method.

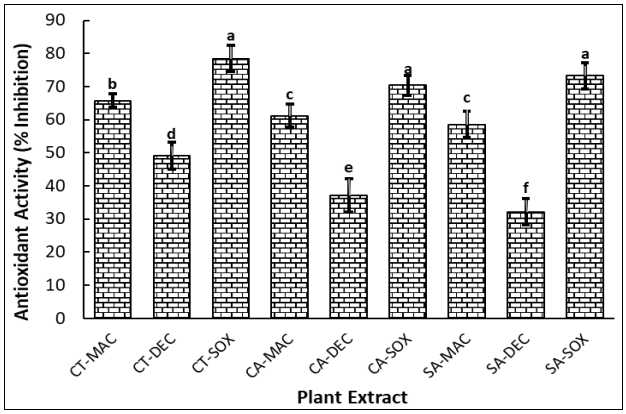

Fig 2: Antioxidant activity (% DPPH inhibition) of plant extracts obtained by different extraction methods. Bars represent mean % inhibition (±SD) of each extract. Different lowercase letters above the bars indicate significant differences at $p<0.01$

4. Discussion

The present study examined the phytochemical content and antioxidant potential of *Clitoria ternatea*, *Cassia tora* and *Sida acuta* using maceration, decoction and soxhlet extraction techniques. CT-SOX showed strong presence (+++) of four phytochemicals (alkaloids, flavonoids, phenolics and tannins) and moderate to weak presence of other phytochemicals which were either very weak or absent in other samples (Fig. 1). Among all treatments, CT-SOX (soxhlet extract of *Clitoria ternatea*) exhibited the highest antioxidant inhibition (78.5 ± 4.1%), followed by SA-SOX (73.3 ± 3.8%) and CA-SOX (70.4 ± 3.5% (Fig. 2). These findings align with earlier reports suggesting that soxhlet extraction is superior in recovering phenolic, flavonoid and alkaloid compounds due to continuous hot solvent percolation (Pradubyat *et al*., 2024; Sathanya *et al*., 2025) [14, 19]. *Ocimum sanctum* extracts obtained *via* soxhlet showed higher phenolic content and stronger DPPH scavenging activity compared to macerated extracts (Chaudhary *et al*., 2020) [5]. Similarly, Kumar *et al*. (2021) [9] demonstrated significantly greater antioxidant efficiency in soxhlet-prepared *Terminalia chebula* extracts. In contrast, decoction extracts (DEC) consistently yielded the lowest values, particularly for CA-DEC (*Cassia tora*) and SA-DEC (*Sida acuta*) which exhibited 37.1 ± 5% and 32.2 ± 4% antioxidant activity, respectively. Qualitative phytochemical analysis of decoction extract of *Cassia tora* and *Sida acuta* showed weak presence (+) to absence (-) of many studied phytochemicals (Fig. 1). Decoction often causes degradation of thermolabile compounds and leaches only water-soluble constituents (Tiwari *et al*., 2011) [21], which limits its efficacy in extracting potent antioxidants such as flavonoids and alkaloids. Similar findings were reported by Veerendrakumar *et al*. (2023) [22] in *Azadirachta indica*, where aqueous decoctions showed lower total phenolic content and reduced antioxidant capacity compared to ethanolic extracts.

Maceration, being a mild cold extraction method, yielded moderate antioxidant values across all three plants. Maceration extract of *Clitoria ternatea* (CT-MAC) and *Sida acuta* (SA-MAC) both performed significantly better as they exhibited respectively 65.8 ± 2% and 58.6 ± 4% antioxidant activity than their respective decoction extracts (i.e. CT-DEC and SA-DEC) but remained less effective than soxhlet extracts (SOX) (Fig. 2). The phytochemical analysis of *Clitoria ternatea* (CT-MAC) and *Sida acuta* (SA-MAC) exhibited moderate presence (++) of nearly all studied phytochemicals. This is consistent with reports suggesting that maceration can extract bioactive compounds without thermal degradation but may not exhaust the material fully (Sasidharan *et al*., 2011) [18]. For *Cassia tora*, CA-MAC outperformed CA-DEC, emphasizing the plant's preference for ethanol-based solvent systems in preserving bioactives such as anthraquinones and glycosides (Sahu *et al*., 2017) [17]. The qualitative phytochemical screening supported these results, with soxhlet and macerated extracts showing strong (++ or +++) presence of flavonoids, phenolics, steroids and alkaloids, compounds widely associated with antioxidant mechanisms (Adawia *et al*., 2016; Nwozo *et al*., 2023) [1, 12]. This qualitative-quantitative correlation reinforces the validity of our experimental design. Compared to existing literature, this study is novel in its simultaneous comparison of three extraction techniques across three botanically unrelated yet pharmacologically relevant plant species. Such cross-species comparison under uniform methodological conditions is scarce and offers new insights into method-plant interactions that optimize phytochemical yield. Overall, the soxhlet extraction was found to be the most effective method for extracting both phytochemicals and antioxidants across all tested medicinal plants, followed by maceration and decoction. The comparative analysis based on qualitative intensity scoring provided insight into the efficiency of each extraction technique in concentrating plant-derived bioactive constituents.

5. Conclusion

The comparative analysis clearly indicates that soxhlet extraction is the most effective method in terms of phytochemical recovery and antioxidant activity across *Clitoria ternatea*, *Cassia tora* and *Sida acuta*. Maceration yielded moderate results, while decoction showed limited efficacy, likely due to compound degradation and lower solubility in water. Among the studied species, *Clitoria ternatea* (CT-SOX) and *Sida acuta* (SA-SOX) displayed superior antioxidant potential, indicating their potential for therapeutic applications targeting oxidative stress. This study not only validate previous findings but also contribute a novel comparative framework, paving the way for more refined extraction protocols in medicinal plant research. However, further work is needed to evaluate different extraction methods and their impact on phytochemical yield, bioactivity, and overall therapeutic efficacy.

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