RESEARCH PAPER

Bioactive Profiling And Extraction Optimization Of (Dhatura) Datura Metel And Tephrosia Purpurea

Shatakshi Kumari[1], Vivek Pratap Singh[2*]

[1]Department of Microbiology, Nims Institute of Allied Medical Science and Technology, Nims University, (Jaipur, Rajasthan), India 303121

[2*]Department of Biotechnology, Nims Institute of Allied Medical Science and Technology, Nims University, (Jaipur, Rajasthan), India 303121

***Corresponding Author:** Dr. Vivek Pratap Singh

Email: vivek.pratap@nimsuniversity.org

Abstract:

Phytochemical studies on bioactive compounds of some medicinal plants are essential for identifying active principles responsible for their medicinal properties and curative uses. *Datura metel* from crude extract of *Datura metel* petroleum ether (PE) and ethanol (E) extracts of its aerial parts β-sitosterol and stigmasterol were isolated whereas chloroform (Ch) extracts afforded daucosterol. Soxhlet extraction was performed using ethanol. Qualitative and quantitative studies of petroleum ether, ethanol and chloroform extracts of crude and Soxhlet extracted *Datura metel* and *Tephrosia purpurea* were conducted. Results of the qualitative study revealed the presence of secondary metabolites of plant origin, including alkaloids, flavonoids, tannins, saponins and glycosides in crude extracts of *Datura metel* and *Tephrosia purpurea*. Spot intensities in purified extracts were increased. Total phenomenon (TPC) and total flavonoid content (TFC) of Soxhlet extracts of *Datura metel* and *Tephrosia purpurea* were increased. *Datura metel* contained higher TPC (80 mg GAE/g) and TFC (62 mg QE/g) than *Tephrosia purpurea* which contained 76 mg GAE/g TPC and 60 mg QE/g TFC. Good extraction yield was achieved from all the batches of plant extract treated. The resultant bioactive phytochemical rich extract after the treatment process contained a powerful group of bioactive phytochemicals comprising of antioxidants and anti-inflammatories that can scavenge for oxidative stress and inhibit the two major enzyme pathways (COX and LOX) implicated in inflammation. The extract also contained saponins and tannins that make a great skin hydrator as well as antimicrobial activity and alkaloids that acts as analgesics. The study, therefore, examines both the qualitative intensity of the various phytochemicals in the treated plant extract as well as the quantitative phytochemicals composition of the bioactive-rich extract obtained. The results underscore Soxhlet extraction as an effective method for improving the recovery of bioactive compounds. This lays the groundwork for creating a synergistic topical formulation that uses Aloe barbadensis (Aloe Vera) as a therapeutic gel base, aimed at treating eczema and related skin conditions.

Keywords: Phytochemical screening, Datura metel, Tephrosia purpurea, total phenolic content, total flavonoid content,

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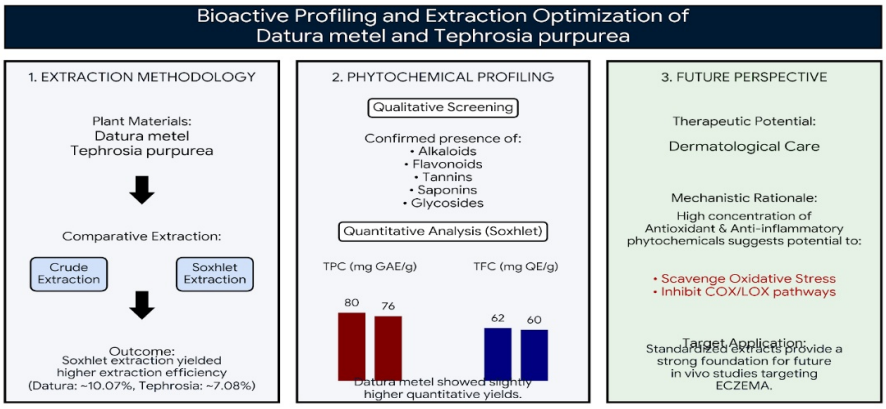
Graphical Abstract:



Introduction:

Ethnobotany, being an offspring of classical ethnology, assumes paramount importance in modern era, in terms of armchair travelling with help of folk literature. It also helps in search out lead compounds for drugs from indigenous plant drugs by analyzing them. Lead plant theory of Julian M. Steward supports the same cause [1-2]. Ethnomedicine is very important as demand for pharmaceuticals is on the continuous increase. Phytochemical analysis is an essential aim in making ethnobotanical and pharmacogenetic studies meaningful. The environment is so wonderful that it has access to amazing variety of compounds of medicinal importance, out of which majority of them are of plant origin [3-4]. In the last few decades, several phyto-constitutes have gained importance due to their diverse structures and a large spectrum of medicinal actions. Therefore, the study provides scope for

*Author for Correspondence: vivek.pratap@nimsuniversity.org

development of newer drugs having safer profile over synthetic analogues [5-6]. The present study deals with identification of some bioactive constituents of two very important ethnomedical plants, namely *Datura metel* (Family: Solanaceae) and *Tephrosia purpurea* (Family: Fabaceae). *Datura metel* (Family: Solanaceae), locally known as Dhatura has been used in various forms to cure diseases for centuries. *Tephrosia purpurea* (Family: Fabaceae), locally known as Sarpunkha is important herbal medicine. Both the plant species are used in traditional folk medicine including indigenous system of Ayurveda for the treatment of various diseases including inflammatory and skin diseases and hence demand for a thorough phytochemical study besides the age-old use [7-9].

1.1 Dermatological determining and Eczema

Chronic skin diseases are now a significant global health and social issue. Atopic dermatitis (eczema) is the most prevalent skin condition, affecting around 10% of children globally and 1–3% of adults worldwide. Chronic itching is a hallmark symptom of eczema, which is characterized by skin inflammation and drying, cracks or fissures in the skin, and increased permeability of skin barriers [10-11]. Atopic dermatitis is distressing and has a major economic and social impact on patients and their families. Eczema currently has no permanent cure, but its effects can be reduced with suitable treatments. Atopic dermatitis is a chronic condition influenced by various factors, including genetic, immune, and environmental elements that contribute to its development. Its pathogenesis has been linked to oxidative stress caused by heightened reactive oxygen species (ROS) and ongoing inflammation driven by elevated pro-inflammatory cytokines and chemical mediators [12-13]. Even though standard treatments for eczema symptoms—such as topical corticosteroids, oral antihistamines, systemic immunosuppressants, and phototherapy—provide symptomatic relief, they come with long-term side effects like atrophy, skin irritation, hormonal imbalances, and systemic toxicity [14]. Safe, effective and economical therapies are urgently needed. Despite the common belief that standard treatments are the sole choice, an increasing amount of evidence suggests that herbal remedies present a multi-faceted strategy with reduced adverse effects and may serve as a comprehensive treatment for schizophrenia [15].

1.2 Phytochemicals' Pharmacological Role

Datura metel and *Tephrosia purpurea* are rich in phytochemicals with a wide array of biological activities. This study addresses the identification of phytoconstituents from the mentioned plants. These plants contain bioactive compounds such as flavonoids, alkaloids, tannins, saponins, glycosides, and terpenoids. The presence of flavonoids and other phenolic compounds endows most of these constituents with antioxidant and anti-inflammatory properties [16-18]. These substances serve as free radical scavengers, thereby reducing oxidative stress—a key contributor to inflammatory skin diseases. These compounds also modulate several metabolic pathways which are involved in skin inflammation. Phytochemicals that inhibit Cyclooxygenase (COX) and Lipoxygenase (LOX) enzymes prevent the production of pro-inflammatory eicosanoids such as prostaglandins and leukotrienes which induce inflammation and cause eczema-like skin symptoms [19-20]. Other compounds such as tannins have been shown to have astringent and antimicrobial properties, whilst saponins are known to increase skin penetration allowing other nutrients to more readily penetrate the skin. With eczema exhibiting such a diverse range of symptoms it is no surprise that there are so many plant derived treatments available [21].

1.2 Plant Profiles and Research Aims

Datura metel, a medicinal plant found in many areas, is also utilized in rituals and religious events. Its application as anti-inflammatory, analgesic and antiseptic is well known. It is also used to cure asthma, cut wounds, ulcers, eczema and other skin diseases. It contains some bioactive secondary metabolites like alkaloids and phenolic compounds, which render it as promising medicinal plant with enormous pharmacological applications. *Tephrosia purpurea* is recognized for its hepatoprotective effects, as well as its notable antioxidant, antimicrobial, and anti-inflammatory properties [22-23]. The flavonoids and terpenoids present in the plant are mainly responsible for these activities. Comparative phytochemical studies of crude and Soxhlet extracted *Datura metel* and *Tephrosia purpurea.* Assessment of increased phytochemical yield and activity of Soxhlet extracted plant extracts. Phytochemical intensity profile of crude and Soxhlet extracted samples by qualitative screening. Quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) of plant extracts. Correlation study of qualitative phytochemical intensity profile with quantitative analysis (TPC & TFC) and dermatological activity (eczema). To enhance the dermatological efficacy of these extracts in future formulations, it is recommended to use Aloe barbadense (Aloe Vera) as the vehicle. Aloe vera is internationally acknowledged for its natural abilities to soothe, hydrate, and aid in wound healing. The hypothesis is that incorporating the enriched phytochemical extracts of D. metel and T. purpurea into an Aloe vera gel matrix will produce a synergistic therapeutic effect that enhances skin barrier repair and provides structural support for treating eczematous conditions. Study the correlation of increased activity of plant extracts with the efficiency of extraction method and to establish scientific basis for the formulation of dermatological preparations from plant extracts. Compoundsified compounds may aid in the development of natural products research as well as the incorporation of traditional health practices into the present health care system.

2. Materials and Methods

2.1 Plant Collection and Authentication

Plant material of medicinal importance was collected from Forest Division Achrol. *Datura metel* (Family: Solanaceae) commonly known as Dhatura and

Tephrosia purpurea (Family: Fabaceae) commonly known as Sarpunkha are used in traditional folk medicine for skin diseases and inflammation. Fresh plant material was collected at time of maximum growth. The collected plant was cleaned up from soil etc. Herbarium sheets of these plant materials were prepared by shade drying followed by making herbarium specimens, mounting and labeling the specimens for taxonomic verification. All the collected plant materials for present research study were taxonomically identified and authenticated from Department of Botany, University of Rajasthan, Jaipur. The submitted herbarium sheets of studied plant species were identified, described morphologically by taxonomist and provided with authentic voucher number of herbarium voucher sheets. The reference number of authenticated herbarium voucher sheets for *Datura metel* and *Tephrosia purpurea* are RUBL21680 and RUBL21682 respectively. These voucher numbers will be used as permanent record and reference numbers for further use to authenticate the plant materials used in present research study.

2.2 Sample Processing of Plant Material

For the present study some important plant parts were selected taking into consideration their common uses and richness of phytoconstituents. Leaves of *Datura metel* (Linn.) leaves and of *Tephrosia purpurea* (Linn.) roots were chosen. Plant parts after washing with running tap water to remove dirt etc. were surface washed with distilled water and allowed to dry in shade at room temperature. No phytoconstituents lose their thermolabile components, which are not exposed to sunlight. Plant materials were completely air-dried then processed using an electric grinder into a coarse powder. The powdered samples were then placed in bags and stored in sterile, sealed containers to keep moisture, oxidation and cross contamination to a minimum. Samples were stored until required for extraction.

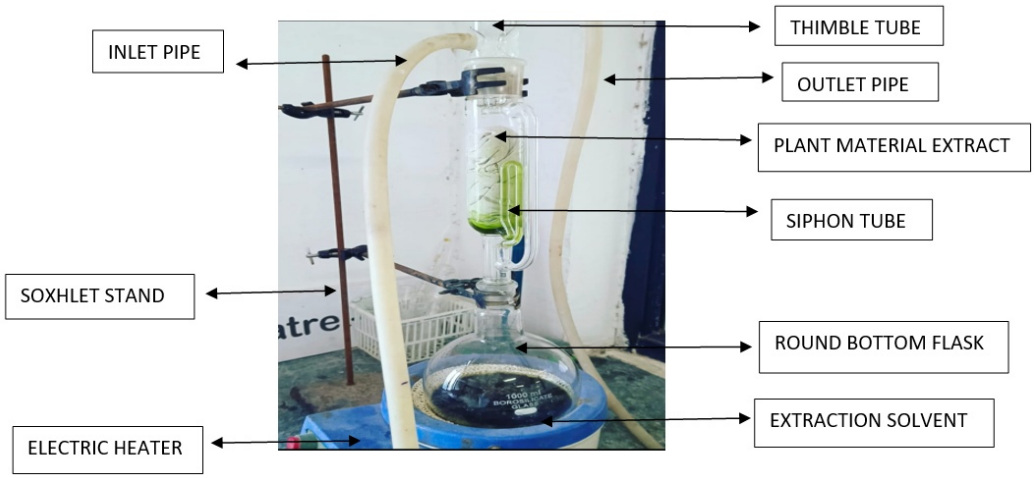
2.3 Preparation of Plant Extracts

The plant extracts were obtained by Soxhlet extraction method and mixed with water and 80% ethanol solution at a ratio of 90:10**.** It is an effective method to extract bioactive compounds from the plant material. Soxhlet extraction involves extracting sample with a solvent in a stream of steam, then recycling the solvent.

2.3.1 Soxhlet Extraction Method

Dried plant samples were ground into powder and extracted using a Soxhlet apparatus with ethanol as the appropriate extraction solvent. This solvent is sufficiently polar to extract a wide variety of phytochemicals such as glycosides, phenolics, alkaloids, and terpenoids from plant samples**.** The experiment was conducted at a temperature of approximately 70°C by filling a glass flask of about 150 ml with ethanol and placed on a heating mantel in the extractor chamber. The specimen was placed in the powdered form in a muslin cloth bag and tied before placing it in the thimble. The vapors of ethanol that evaporated from the solvent in the flask were condensed in a water-cooled condenser and passed through the plant specimen (Fig. 1). The extraction was performed using a 1:2 (w/v) solvent-to-sample ratio. A complete extraction of all phytoconstituents from the raw sample was achieved using a multiple cyclic solvent reflux technique. By means of evaporation, the extract was used to eliminate the resulting solution, which produced a concentrated liquid solution. This solution was provided in a semi-solid or dried state, hereafter referred to as a "pure extract." The weight of the pure extract was recorded and then stored in a desiccator to minimize exposure to moisture.

Fig 1. Preparation of Drugs using the Soxhlet apparatus



2.4 Determination of Extraction Yield

The efficiency of the extraction processes was evaluated in terms of percentage yield of the resulting extracts. The yield of extraction is an indicative parameter of the phytoconstituents extracted from the plant material.

$$\text{Yield (\%)} = \left(\frac{\text{Weight of Pure Extract}}{\text{Weight of Crude Extract}}\right) \times 100$$

calculations were performed for each of the experiments to compare the efficiencies of Soxhlet extraction as well as to compare the efficiency of the crude extracts' extraction to the purified botanical extracts.

2.5 Statistical Analysis

The outcomes are presented as the mean ± SD of 'n' determinations based on a minimum of triplicate samples. One-way ANOVA was used to compare the results. Significantly different results are indicated by (*) $p < 0.05$ or (**) $p < 0.01$ from the ANOVA test., the means of individual groups were compared using Tukey's test. Extracts prepared by Soxhlet extraction showed higher bioactivity than crude extracts.\

Table1. Drug preparation of *Datura metel* according to Batch.

BATCH	*DHATURA METEL*(L)CRUDE EXTRACT (gm)	ETHANOL (ml)	SOXLET TIME	PURE EXTRACT (gm)	Extraction Yield (%)
B1	50 g	100 ml	10:00hrs	5.02 ± 0.05	10.04 ± 0.10
B2	50 g	100 ml	9:45hrs	4.99 ± 0.04	9.98 ± 0.08
B3	50 g	100 ml	10:15hrs	5.06 ± 0.06	10.12 ± 0.12
B4	70 g	150 ml	11:00 hrs	7.07 ± 0.07	10.10 ± 0.11
B5	60 g	130 ml	10:30 hrs	6.07 ± 0.06	10.12 ± 0.09

Fig 2. Pure Extract of *Dhatura metel* according to Batch

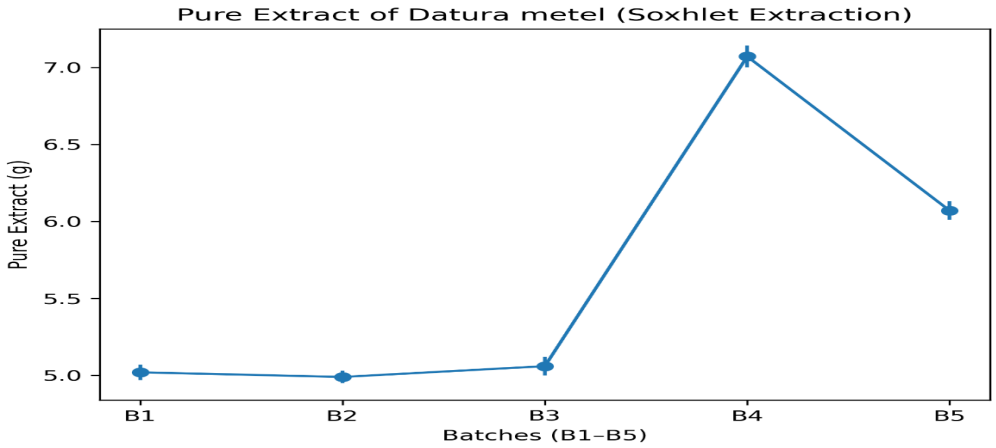
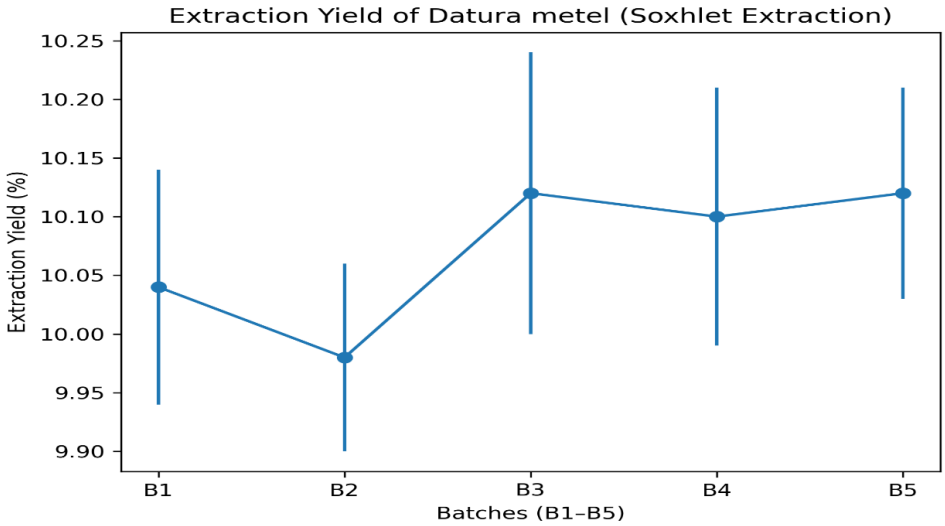


Fig 3. ExtractionYield of *Dhatura metel* according to Batch



Soxhlet extraction experiments were performed using leaves of *Datura metel* for different batches. The results obtained for different batches i.e., B1–B5 were similar. For batches B1–B3 of 50 g of *Datura metel* , using 100 ml of ethanol, 4.99–5.06 g of extract was obtained with 10% efficiency. An increase of 20% in weight of sample by making B4 (70 g) and B5 (60 g) did not change the percentage of yield of extract; the extract obtained was 7.07 g and 6.07 g, respectively. The efficiency of extraction remained 10%. The process was reproducible.

Table2. Drug preparation of *Tephrosia purpurea* according to Batch.

BATCH	*TEPHROSIA PURPUREA* (R)CRUDE EXTRACT (gm)	ETHANOL (ml)	SOXLET TIME (hours)	PURE EXTRACT (gm)	Extraction Yield (%)
B1	25g	75 ml	7:45 hrs	1.77 ± 0.03	7.08 ± 0.07
B2	50 g	100 ml	9:50 hrs	3.48 ± 0.05	6.96 ± 0.06
B3	50 g	100 ml	10:00 hrs	3.60 ± 0.06	7.20 ± 0.08
B4	60 g	130 ml	10:45 hrs	4.25 ± 0.07	7.08 ± 0.07
B5	50 g	100 ml	9:55 hrs	3.55 ± 0.05	7.10 ± 0.07

Fig 4. Pure Extract of *Tephrosia purpurea* according to Batch.

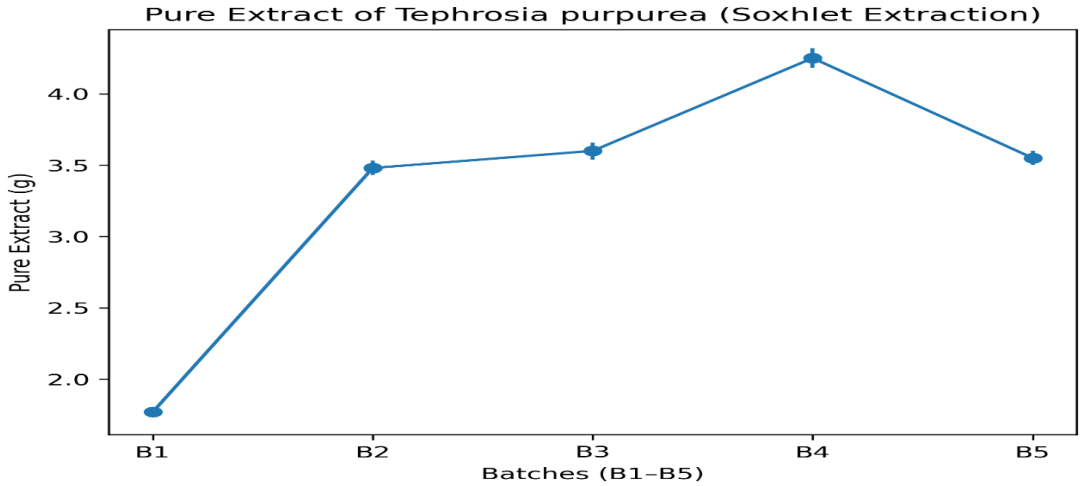
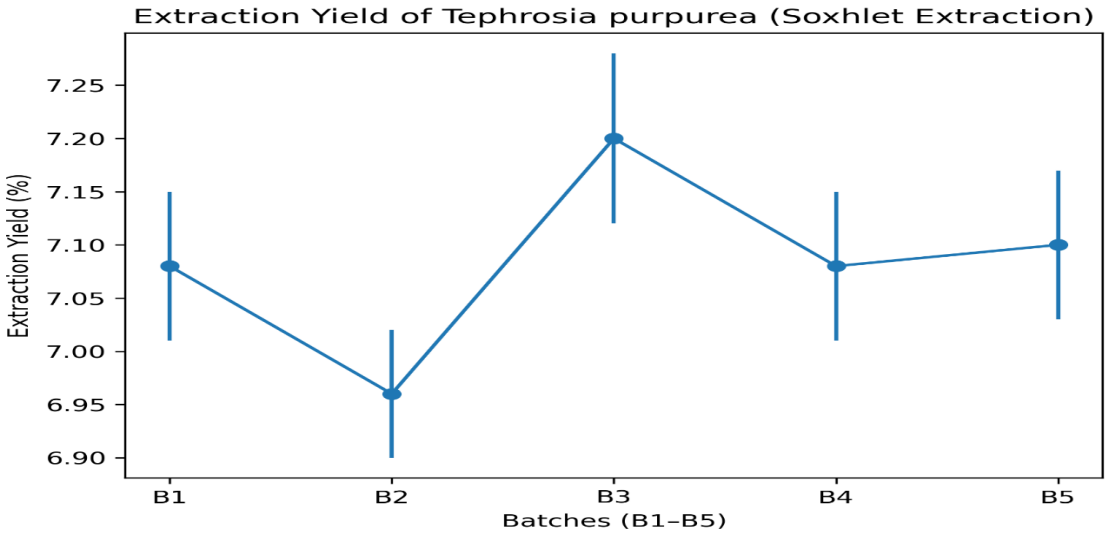


Fig 5. ExtractionYield of *Tephrosia purpurea* according to Batch.



The results indicate uniformity in the process performance from B1 to B5. In B1 (25 g, 75 ml) yielded 1.77 g of extract with 7.08% yield on dry basis. In B2, B3 and B5 (50 g, 100 ml) yielded 3.48–3.60 g of extract with 6.96% to 7.20% yield on dry basis. B4 made 60g of extract which weighed 130ml. After drying it weighed 4.25g and had a 7.08% recovery yield. As with the previous batch, all variables (amount of material, volume of solvent, time etc) affected the weight of the extract but not the percentage of recovery. Overall, the total yield was consistent with our previous results of ~7% (Table 2, Fig. 4,5).

Determination of Total Phenolic Content (TPC)

Total Phenolic Content (TPC) of the studied extracts was determined by a modified Folin-Ciocalteu colorimetric assay. The Folin-Ciocalteu assay is commonly used for the determination of total amount of phenolic compounds in plant extracts. It is based on reduction of Folin-Ciocalteu reagent by phenolic compounds from studied samples under alkaline

conditions, which form a blue-colored chromophore. The color intensity, measured spectrophotometrically, is directly proportional to total phenolic content of the sample. A measured amount of plant extract solution was mixed with Folin–Ciocalteu reagent and a certain amount of sodium carbonate solution in test tube. The reaction started by mixing the solutions. After a definite time for full colour development, the absorbance of the resulting reaction mixture was measured at 765 nm against a blank (distilled water) using UV–Visible spectrophotometer. The total phenolic content was determined using a calibration curve of gallic acid standard solutions and expressed as milligram of Gallic Acid Equivalent per gram of extract (mg GAE/g extract). The use of GAE as a reference allowed a reliable quantification of the samples studied, since the plant extracts are rich in bioactive phenolic compounds with marked free radical scavenging activity.

Determination of Total Flavonoid Content (TFC)

Total Flavonoid Content (TFC) was determined by aluminum chloride colorimetric method. TFC estimation is based on the formation of stable complexes of flavonoids with aluminum ions (Al^{3+}) and expressed as yellow pigments. Quercetin-like content in plant extracts was determined by mixing the sample with aluminum chloride solution and after 24 hours at room temperature measuring the absorbance at 415 nm using UV–Visible spectrophotometer. For calibration standard quercetin was used. Flavonoids are based on a flavanol structure and are expressed as milligrams of Quercetin Equivalent per gram of extract (mg QE/g extract). Flavonoids, and many other polyphenols, function as antioxidants, protecting against oxidative stress. In addition to antioxidant activity, these substances have been shown to have anti-inflammatory and other protective effects on living tissues and can treat a variety of diseases.

Advanced Phytochemical Characterization

For the identification and specification of individual phytoconstituents more precise analytical methods are required. The extract's phytochemicals were identified and quantified with HPLC, using retention time and peak area for each compound. Using Liquid Chromatography–Mass Spectrometry (LC–MS), bioactive compounds were analyzed and identified according to their molecular weight and structure. Improvements in methodology and analytical precision enabled the validation of compounds. The study commenced with initial phytochemical screening of various plant species and in addition, more complex methodologies were employed to establish the chemical profiles of the plants studied. P. crude and pure extracts of *Datura metel* and *Tephrosia purpurea*:

Result

Qualitative Phytochemical Screening

Qualitative analysis for major classes of secondary metabolites was studied for both crude and Soxhlet extracts of *Datura metel* and *Tephrosia purpurea*. Test procedures and characteristics of various standard chemical tests were discussed. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins and glycosides. Identification of phytoconstituents was done by appearance of colour, fluorescence and appearance of precipitate.

Fig 6. Phytochemical analysis of pure extracts of plant materials

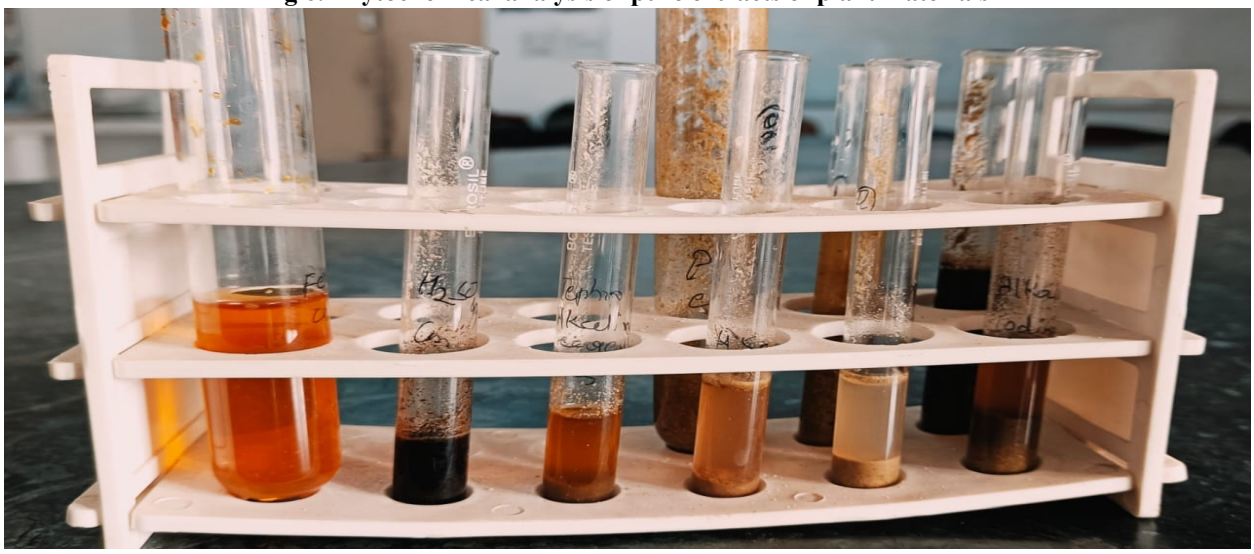


Fig 7. Phytochemical analysis of crude extracts of plant materials

Comparative Phytochemical Analysis (Crude vs Pure Extracts)

A comparative study on bioactive metabolites present in crude and distilled extracts of Fomes commentaries was carried out. Clearly visible color changes, increased gel formation and distinct reaction were observed with pure extracts (Fig. 5) after distillation. An appreciable increase in intensity of bioactive metabolites in Soxhlet distilled extract (Fig. 6 & 7) than in crude extracts were observed.

Phytochemical Findings

The bioactive constituents of the two plant species studied for antifungal activity consisted of several biological active compounds that reacted with standard chemicals on TLC plates. The isolated constituents from the two plant species consisted of alkaloids, flavonoids, tannins, saponins and glycosides. The Soxhlet extracts of the two plant species studied contained a higher concentration of bioactive constituents than the crude plant extracts. The increased efficiency to extraction and yield of biologically active compounds of such kind testifies to the enhanced reaction intensity. The revealed biologically active compounds possess a strong pharmacological activity. They can be used as cosmetic agents and as food additives (for example, as antioxidants) or introduced into dermatological preparations.

Phytochemical Observations of *Datura metel*

A detailed qualitative study of *Datura metel* showed an increased phytochemicals content in the purified extracts. The isolated constituents showed intense reddish-brown color in iodine test as compared to the lighter yellowish-brown color in crude extracts. The flavonoids obtained were found to have bright yellow color and showed enhanced fluorescence under UV light. The tannins gave a well-defined white precipitate in gelatin test. The saponins also gave thick and persistent foam. The crude extracts gave a very feeble test with less persistence and intensity.

Table 3 Comparative study of crude and pure *Datura metel* extracts at phytochemicals test on color, composition etc.

Component	Test Name	Crude Extract Characteristics	Pure Extract Characteristics
Alkaloids	Iodine Test	Yellowish-brown or reddish-brown, less intense.	Distinct reddish-brown, more vivid.
	Picric Acid Test	The resulting product was a light-yellow liquid with a suspended solid.	The resulting bright yellow solid was hard and had a good texture and was very uniform.
Flavonoids	Alkaline Reagent Test	Light Yellow or Faint Fluorescence.	Bright Yellow Color or Strong Yellow Fluorescence Under UV Light
	Ferric Chloride Test	Greenish blue with slight turbidity.	intense green, blue or purple color with good clarity.
Tannins	Gelatin Test	This test indicates whether gelatin is present in a solution.	A gelatin solution is cloudy, it appears turbid or opalescent, and there may be present a faint precipitate. A normal solution remains clear until a very large

			number of solutions is added, at which time it will turn turbid.
	Braymer's Test	Greenish-black solution, slightly turbid.	Intense blue-black solution.
Saponins	Foam Test	the formation time of any liquid tested which can form light foam that disappears quickly	thick and persistent foam that will sit for several minutes, or any degree in between.
	Emulsion Test	Make a milky white and less stable emulsion that separates within a short time.	Consisting of a stable, non-transparent emulsion that remains mixed.
Glycosides	Borntrager's Test	samples became a light pink color with mottling.	The synthetic urine developed a vivid reddish-pink hue all over, particularly within the ammoniacal ring.
	Keller-Killiani Test	faint reddish-brown or blue staining around junction and slightly diffuse	but distinctly a reddish-brown or blue stained well-defined junction between two layers.

Phytochemical Observations of *Tephrosia purpurea*

Phytoconstituents in pure extract *Tephrosia purpurea* were found to be more concentrated than present in crude extracts. Alkaloids from *T. purpurea* on stand deposited as clear yellowish-brown precipitate. The test of flavonoids showed bright green or purple solution of high clarity. Saponins on shake formed opaque emulsion while glycosides by Borntrager's test showed uniform reddish pink color.

Table 4. Comparing Pure and Crude Extracts in *Tephrosia purpurea* Phytochemical Analysis by Components, Color and Appearance:

Component	Test Name	Crude Extract Characteristics	Pure Extract Characteristics
Alkaloids	Iodine Test	The resulting color is a pale yellowish-brown or reddish-brown solution, or a slightly turbid precipitate.	A vigorous color test yields a vivid hue and a precipitate of high intensity.
	Picric Acid Test	Pale yellow color with scattered particles or light sediment.	Vivid yellow precipitates, showing greater uniformity and clarity.
Flavonoids	Alkaline Reagent Test	Pale yellow or very faint fluorescence	Bright yellow color or very bright fluorescence.
	Ferric Chloride Test	Dull green or bluish with some turbidity.	very bright green, blue, or purple with the solution being clear.
Tannins	Gelatin Test	A cloudy suspension with a slight precipitate is produced.	A distinct solid or cloudy precipitate, more pronounced than the above.
	Braymer's Test	fluorescent liquid bleed test that is light green or blue-black and has some dispersion.	The very bright green or blue-black test has a very bright fluorescence, with a sharp and uniform test color.
Saponins	Foam Test	surface active properties like those of soap,	The lather produced by saponins in the Foam Test

		producing a rich lather when mixed with water.	turned light and soon disappeared.
	Emulsion Test	Sees Emily's liquid is a milky emulsion that separates rapidly.	Creates a stable, opaque emulsion that remains intact.
Glycosides	Borntrager's Test	Uneven distribution of pale pink or reddish hues.	Bright reddish-pink color in ammoniacal layer, more uniform.
	Keller-Killiani Test (Water / Glycerol / Ethylene Glycol)	Weak reddish-brown or blue color at the junction slightly diffused.	Strong red-brown or blue color at the junction with a sharp interface.

Table 5. Phytochemical Intensity Comparison

Component	Test Name	Datura (Pure)	Tephrosia (Pure)	Function in Eczema
Alkaloids	Iodine	+++ (Red Brown)	++ (Yellow Brown)	Effects on pain relief
Flavonoids	Alkaline	++ (Bright Yellow)	+++ (Strong UV Fluo.)	anti-inflammatory due to inhibition of cyclooxygenase (COX) and lipoxygenase
Saponins	Foam	+++ (Stable Foam)	++ (Opaque Emulsion)	utilized to produce foam for skin care items that results in a stable foam, a non-transparent emulsion for a cleansing wash, and for aiding in the creation of.
Tannins	Gelatin	+++ (White PPT)	+++ (Solid PPT)	Protection against microbes.
Glycosides	Borntrager's	+++ (Reddish Pink)	+++ (Uniform Pink)	All the known sugars and their bioactive counterparts show bioactive synergy.

Soxhlet extracts of Tectaria truncata were found to have more concentrated phytochemicals than crude extracts and appeared darker in color with a consistent precipitate as opposed to the crude extracts. Phytochemical intensity symbols indicate levels of concentration: +++ = high intensity, ++ = moderate intensity, + = low intensity. All species studied were found to contain all the bioactive compounds in different intensity. *Datura metel* contains +++ alkaloids, +++ saponins, +++ tannins and +++ glycosides whereas *Tephrosia purpurea* contains +++ flavonoids having strong fluorescence along with +++ glycosides. These biological extracts could be due to analgesic activity of alkaloids, anti-inflammatory activity of flavonoids through inhibition of COX/LOX enzymes, skin hydration and moisture by saponins, antimicrobial activity of tannins and synergistic activity of glycosides. Phyto-extracts of these species were purified by Soxhlet extraction and shown excellent concentration of bioactive Phyto-constituents with impurities responsible for less efficacy and non-pharmacological activities. Details regarding the concentration of bioactive components along with pharmacological activities are presented in (Table 3, 4 & 5) .

Interpretational Analysis of Qualitative Screening

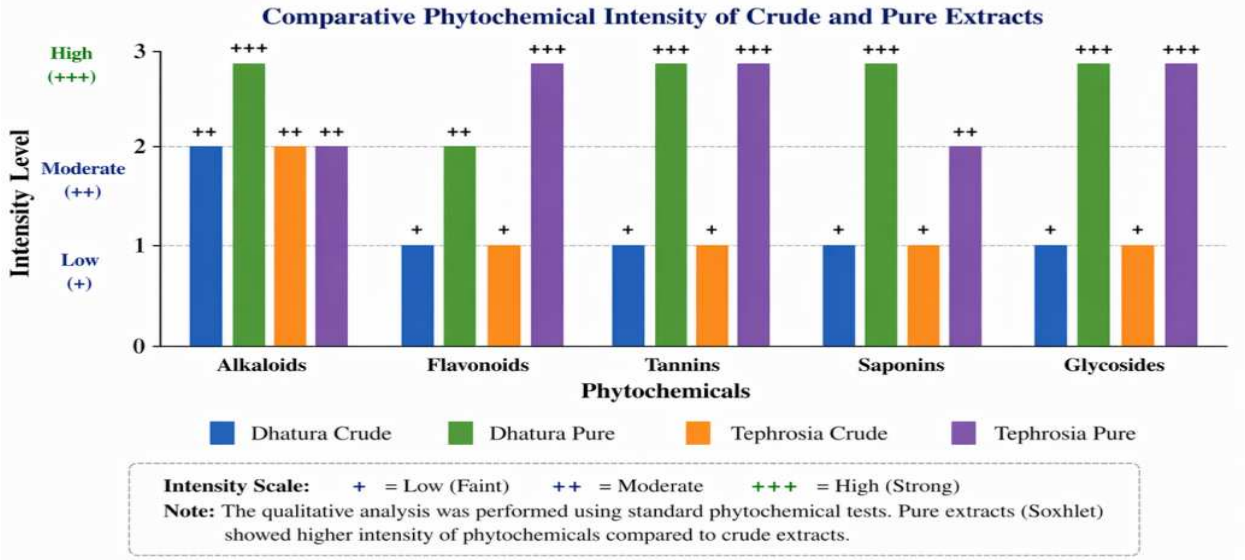
Qualitative phytochemical profiling of the extract showed an overall increase in intensity of the extract components from crude to pure. The Iodine test showed a deep reddish-brown colour produced within a few minutes. This confirmed the presence of high levels of quaternary alkaloids. Persistent stable foam produced in the saponin test indicated the presence of specific glycosidic surfactants which are crucial in maintaining skin hydration in inflammatory skin diseases.

Table 6 & Fig 8. Qualitative Phytochemical Screening and Comparative Intensity of Crude and Pure Extracts; and Comparative Phytochemical Intensity of Crude and Pure Extracts of the Plant parts studied.

Qualitative Phytochemical Screening and Comparative Intensity of Crude and Pure Extracts

Compound	Dhatura Crude	Dhatura Pure	Tephrosia Crude	Tephrosia Pure
Alkaloids	++	+++	++	++
Flavonoids	+	++	+	+++
Tannins	+	+++	+	+++
Saponins	+	+++	+	++
Glycosides	+	+++	+	+++

Intensity Scale: + = Low (Faint) ++ = Moderate +++ = High (Strong)



Intensity Scale: + = Low (Faint) ++ = Moderate +++ = High (Strong)
Note: The qualitative analysis was performed using standard phytochemical tests. Pure extracts (Soxhlet) showed higher intensity of phytochemicals compared to crude extracts.

(Table 6 and Fig. 8) depicted qualitative phytochemical analysis of *Datura metel* and *Tephrosia purpurea* (crude and pure extracts). (+) low, (++) moderate and (+++) high intensity of phytochemicals have been shown in table 6. The intensity of phytochemicals in pure extracts is increased than crude extracts due to increased solvent penetration, efficient disruption of complex plant cellular structures and extraction of bioactive compounds. A consistent increase in intensity of all phytochemicals from crude to pure extracts of test plants depicted uniform and efficient extraction. Intensity of phytochemicals in both plant extracts was higher than their corresponding crude extracts. Visible Spectrum's data for *Datura metel* reveals the presence of (+++) alkaloids, tannins, saponins and glycosides in the pure extract. The crude extract of *Datura metel* reveals low to moderate presence of the same. For *Tephrosia purpurea*, visible Spectrum's data reveals high (+++) presence of flavonoids, tannins and glycosides in the pure extracts. The crude extracts of Tephrosia purpurea reveal low presence of these phytochemicals. Intensity of these phytochemicals is higher in *Datura metel* as compared to *Tephrosia purpurea*. There is species specific variations in the phytochemicals present in these extracts. Fig. 8 Results illustration in bars. The height of the bars corresponding to the pure extracts were higher than those of the crude extracts. All the pure extracts height were uniformly higher than the corresponding crude extracts. This result confirms that Soxhlet extraction method is efficient in producing bioactive phytochemicals responsible for the increased pharmacological activities such as anti-inflammatory, antioxidant and antimicrobial activities. Hibiscus contains high levels of flavonoids, tannins and glycosides meaning it is a fantastic natural emollient and skin soother. These natural ingredients have strong antioxidants, anti-microbial and anti-inflammatory properties, making them very effective for treating Eczema. They Neutralize the body's oxidative stress (ROS) production and reduce the body's production of pro-inflammatory enzymes (Cox and Lox) that cause the symptoms of eczema. These natural ingredients work to repair the skin's natural barrier function so the skin can return to normal naturally during an outbreak. Quantitative Phytochemical Analysis (TPC & TFC) Phenolic and flavonoid contents of *Dhatura metel* (D.m) and *Tephrosia purpurea* (T.p) were found higher in pure extract than in crude extract. The pure extract of D.m showed the highest Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) than T.p. An increase in phytochemicals was observed after Soxhlet extraction process.

Comparative Analysis:

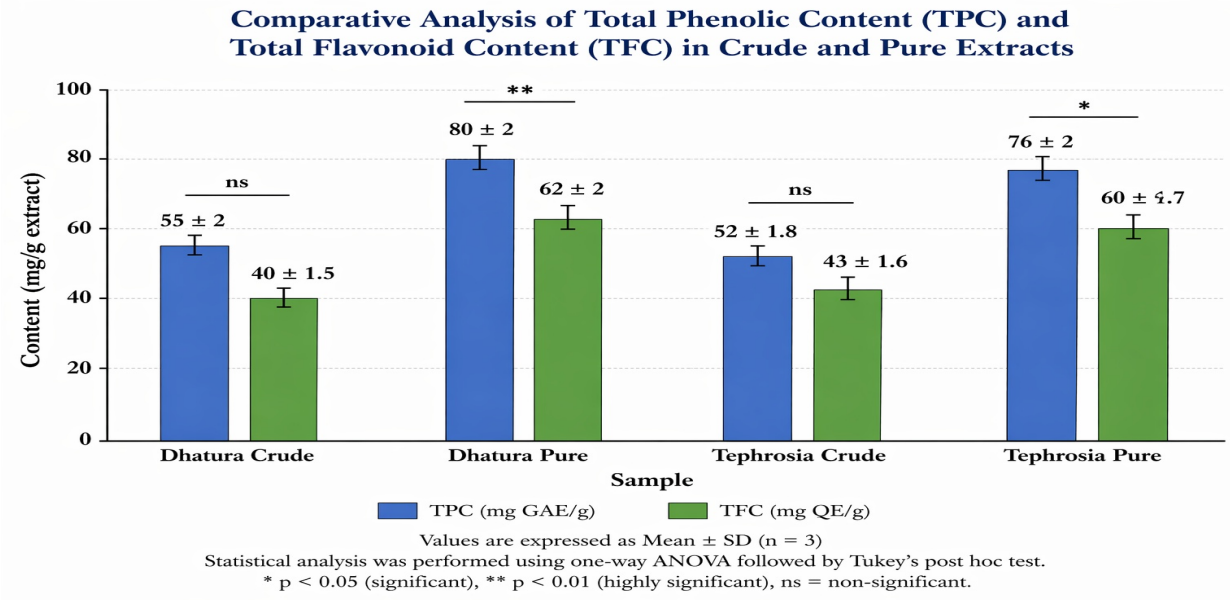
Dhatura metel is very effective phytochemical rich source as it has higher content of alkaloids and phenolics and therefore more bioactive than *Tephrosia purpurea*. % extraction efficiency for *D. metel* (7.23%) was only marginally more than *Tephrosia purpurea* (6.78%) (Table 7, Fig 9).

Table 7. Total Phenolic and Flavonoid Content in crude and pure extracts

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	Significance
Dhatura Crude	55 ± 2	40 ± 1.5	ns
Dhatura Pure	80 ± 2	62 ± 2	**
Tephrosia Crude	52 ± 1.8	43 ± 1.6	ns
Tephrosia Pure	76 ± 2	60 ± 1.7	*

Quantitative densitometric studies of extract revealed highly significant ($p < 0.01$) increase in TPC and TFC for *D. metel* and significant ($p < 0.05$) increase in TFC for *T. purpurea*. The difference between crude and extracts of both plant materials were found to be non-significant (ns). This indicates that the concentration of bioactive molecules in crude extracts of plant materials are low and inconsistent due to impurities and cellular debris present in it which gets extracted out during exhaustive process of Soxhlet extraction.

Fig 9. Comparative Examination of Total Phenolic Content (TPC) and Total Flavonoids Content (TFC) in Crude versus Pure Extracts



In the present investigation, there was a noticeable increase in Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in pure extracts as compared to crude extracts. *Dhatura metel* exhibited highly significant increase (**$p < 0.01$) in TPC and TFC whereas *Tephrosia purpurea* showed significant increase (*$p < 0.05$). TPC and TFC were determined to use respective standard solutions of gallic acid and quercetin and expressed as milligram of gallic acid equivalent (GAE) and quercetin equivalent (QE) per gram of extract.

Discussion

A comparative study on crude and Soxhlet extracts of *Datura metel* and *Tephrosia purpurea* were carried out. The extracts of both the species showed increase in phytoconstituents and quality of extracts. These findings are in accordance with previous findings which indicated that continued extraction by solvent cycling in Soxhlet apparatus is effective for recovery of bioactive compounds than conventional method of extraction. Utilizing Aloe barbadensis to incorporate these concentrated phytoconstituents into a topical gel serves two purposes for effectively delivering them to the affected epidermal layers. In addition to functioning as an effective, biocompatible delivery vehicle, the Aloe vera base provides intrinsic anti-inflammatory and moisturizing effects that are vital for thorough eczema management.

Phytochemical Enrichment and Comparative Efficiency

Qualitative screening on crude and purified extracts of medicinal plants showed enhanced color intensity and stable precipitates. This observation is in consonance with existing literature regarding extraction of medicinal plants using Soxhlet extraction technique. The method facilitates solubilization and recovery of bioactive Phyto-constituents like phenolics and flavonoids from plant matrix by effective penetration of solvent and repetitive extraction. The % yield of phytoextract obtained from *Datura metel* was ~10.07%,

and for *Tephrosia purpurea* was ~7.08%. This may be due to the difference in the matrices of the two plants. The solvent might penetrate and solubilize the phytoconstituents from the higher porosity of leaf matrix of *Datura metel* more effectively than from the lignified root matrix of *Tephrosia purpurea*. Thus, *Datura metel* is found to be a better matrix than *Tephrosia purpurea* for extraction of phytoconstituents.

Phytochemical Dominance and Scientific Interpretation

Species wise comparative profile of different phytoconstituents showed that each species has its own set of phytochemicals. *Datura metel* was found to be richer in alkaloids and saponins whereas *Tephrosia purpurea* was rich in flavonoids. This could be due to species specific metabolism or selection of plant material. It has been observed that the leaves of most medicinal plants accumulate higher levels of alkaloids and phenolics due to their active photosynthetic metabolism, while the roots of these species contain fewer extractable phytochemicals, which are often structurally bound. impact the results of extraction processes, and therefore, choosing suitable plant parts for extraction is essential. Although some studies have shown differences in bioactive compound content among leaves, flowers, seeds, roots, and other plant tissues, such information is essential for determining optimal strategies and selecting appropriate materials for extraction processes.

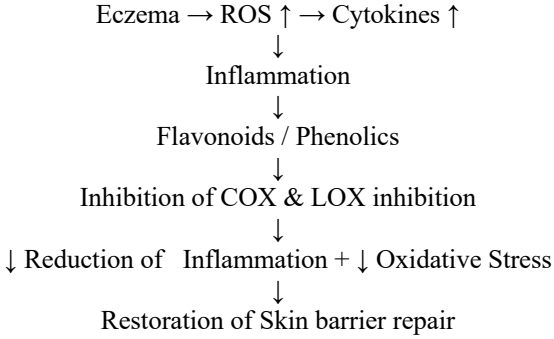
Mechanistic Significance in Dermatological Applications

The results of the qualitative bioactivity assays were further corroborated by the quantification of TPC and TFC. The Soxhlet extract was found to contain higher amounts of bioactive compounds such as phenolic and flavonoid that can be used for the treatment of eczema. The active constituents of Neem tree like flavonoids and phenolics have antioxidant activity since they contain free radical scavengers. Some of the antioxidants in Neem also inhibit the enzymes cyclooxygenase (COX) and lipoxygenase (LOX) which cause inflammation. Saponins in Neem also help in skin hydration and permeability. Tannins have antimicrobial activity while alkaloids in Neem have analgesic activity. The product has a synergistic effect of all the therapeutic active constituents present in Neem.

Critical Insight and Limitations Integration

While the qualitative intensity assessment did not identify any bioactive compounds as positive, this study had several limitations. First, the bioactive qualitative intensity assessment relied upon a visual interpretation of color reactions that may be very subjective. Second, detailed identification of bioactive compounds that were positive in the bioactive screen were not conducted using more sophisticated methods, such as HPLC or LC-MS. In vitro and in vivo validation of bioactivity of the extract were also not done in this study. However, there was a significant increase in both qualitative intensity and in quantitative parameters like TPC and TFC along with their significance tests. Further applications of these strategies to synthesize, investigate further compounds, validate the generated mechanisms of action and formulations for clinical use would be promising.

Fig 10. Suggested Mechanism of Anti-inflammatory Effect



(Fig10.) Anti-inflammatory action of phytochemicals interfering with the COX and LOX pathways, reducing oxidative stress and repairing the skin barrier.

Clinical Implications

The extracts of Datura metel (DM) and Tephrosia purpurea (TP) obtained through Soxhlet extraction show significant potential as thermotherapeutic agents. DM and TP both demonstrated significant levels of flavonoids and phenolics, which function as antioxidants and anti-inflammatory agents by inhibiting key enzymes (COX and LOX). The extracts also contained saponins and tannins, which aid in skin hydration. TP has anti-irritant components, whereas DM contains alkaloids in its extract that serve as an anti-irritant and contribute to skin care. Creams and gels can be formulated with these plant extracts for topical use.

Limitations

Color reactions are often subject to considerable qualitative bias and in this survey were not backed up by more sophisticated techniques such as HPLC or LC-MS mass spectroscopy to establish positive

identification of the compounds concerned. Bioassays to establish efficacy were not undertaken. Variation sources (both environmental and experimental), sample size, and safety assessment were not considered.

Future Scope

It is necessary to conduct additional studies employing advanced analytical methods like HPLC and/or LC-MS, as well as further biological validation and mechanism of action research. Future studies will focus on creating and physically characterizing (including properties such as pH, spreadability, and viscosity) a topical gel based on Aloe barbadensis that includes these enriched extracts derived from Soxhlet. To validate the suggested synergistic effectiveness, safety profile, and applicability on a large scale, it is necessary to develop this optimized delivery system and conduct in vitro and vivo clinical assessments.

Conclusion

Phytochemicals were obtained by Soxhlet extraction from the plant materials Datura metel and Tephrosia purpurea. Both crude and Soxhlet extracts of the studied species, *Datura metel* and *Tephrosia purpurea*, showed increase in both qualitative and quantitative bioactive compounds like alkaloids, flavonoids, tannins, saponins and glycosides. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of both studied species increased with high degree of significance. *Datura metel* showed better extractives % and Phyto-constituents intensity whereas, *Tephrosia purpurea* gave flavonoids with other Phyto-constituents as major constituents. The Phyto-constituents of these study extracts were found to possess antioxidant, anti-inflammatory, antimicrobial and skin protective effects. These natural products could be one of the most promising therapeutic alternatives for the treatment of eczema. The extraction technique and its optimization is one of the keys to increasing the concentration of Phyto-constituents and bioactive compounds from natural resources. Soxhlet extraction technique was found most suitable for the extraction of Phyto-constituents due to its simplicity, feasibility and applicability for different natural matrices. The high extraction yield and strong presence of COX/LOX inhibiting phytochemicals demonstrate that these standardized extracts hold significant potential for future dermatological studies, particularly in the treatment of eczema.

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