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ORIGINAL ARTICLE **OPEN ACCESS**

Assessment of Anti-Inflammatory Effect of *Permoterma reticulatum, Curcuma caesia and T. patula* using *in-vitro* study

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ABSTRACT
The goal of the current study was to assess the anti-inflammatory effects of Permoterma reticulatum, Curcuma caesia and T. patula using phytochemical test using in-vitro protein denaturation methods. The in-vitro % inhibition of protein denaturation method was used to study and assess the anti-inflammatory properties of Permoterma reticulatum, Curcuma caesia and T. patula extracts. In order to evaluate the anti-inflammatory property, the test extracts were incubated with egg albumin at various concentrations under strictly controlled experimental circumstances. The reference medication utilised was acetyl salicylic acid. The current findings showed that hydroalcohalic extracts of Curcuma caesia exhibited highest inhibited protein (albumin) denaturation in a concentration-dependent manner. In the normal group, standard group, hydroalcohalic extracts of Permoterma reticulatum, Curcuma caesia and T. patula, the percentage inhibition of proteinase activity of Curcuma caesia was found to be 84.8%, Permoterma reticulatum 59.1%, 36.2%, respectively. All things showed that hydroalcohalic extracts of Curcuma caesia and Permoterma reticulatum having strong anti-inflammatory bioactive components and that can aid to lessen inflammation and fend off chronic illnesses. To get the most anti-inflammatory benefits from Camellia sinensis, however, additional research is required to discover the ideal dosage and time frame for consumption. The findings of this research offer insightful information about the phytochemical makeup and anti-inflammatory activities of Curcuma caesia, which may be helpful in the creation of innovative therapeutic agents for the treatment of chronic inflammatory illnesses.
Keywords: *Camellia sinensis, anti-inflammatory, herbal plants, Thin layer chromatography.*

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INTRODUCTION

Medicinal plants have been used for thousands of years to treat various health conditions and diseases. They contain active compounds that have therapeutic properties and can be used to promote healing and alleviate symptoms. Different parts of the plant may contain different active compounds, such as the leaves, flowers, roots, or bark. Medicinal plants can be consumed in various forms, including teas, tinctures, capsules, and extracts.[1,2] In recent years, there has been a growing interest in natural remedies and alternative medicine, leading to increased research on the medicinal properties of plants. Many modern medicines are derived from medicinal plants, and scientists continue to study the potential of these plants for future medical applications. However, it is important to note that not all medicinal plants are safe and effective for everyone. Some may interact with medications or cause side effects, and their use should always be discussed with a healthcare provider. Overall, medicinal plants offer a natural and potentially effective alternative to conventional medicine, and their use has been an important part of traditional medicine for centuries.[3]

Inflammation is a biological response of the body's immune system to harmful stimuli such as pathogens, damaged cells, or irritants. The main purpose of inflammation is to protect the body by eliminating the initial cause of cell injury, clearing out necrotic cells and tissues damaged from the original insult, and initiating the tissue repair process. [4] The inflammatory response involves the activation of immune cells, such as white blood cells and cytokines, which release chemicals that increase blood flow to the affected area and cause swelling, redness, heat, and pain. This response is part of the body's natural defense mechanism against infection and injury, but chronic inflammation can lead to tissue damage and disease. [5] Inflammation can occur in response to various factors such as infections, injuries, allergies,

autoimmune disorders, and exposure to toxins. Common symptoms of inflammation include redness, swelling, warmth, pain, and loss of function in the affected area. Treatment of inflammation typically involves addressing the underlying cause and may include medications such as anti-inflammatory drugs, corticosteroids, or immunosuppressants.[6] Inflammation is a natural response of the immune system to protect the body against harmful stimuli, such as pathogens, injuries, or toxins. There are two main types of inflammation: acute inflammation and chronic inflammation [7]. Acute inflammation: This type of inflammation is a short-term response that occurs rapidly after injury or infection. It is characterized by the classic signs of inflammation, including redness, swelling, heat, pain, and loss of function. Acute inflammation is a necessary part of the healing process and helps to clear the injured or infected tissue of debris and pathogens [8]. Chronic inflammation: This type of inflammation is a long-term response that persists even after the initial cause of the inflammation has been resolved. Chronic inflammation is often associated with a range of diseases, including arthritis, diabetes, heart disease, and some types of cancer. Unlike acute inflammation, chronic inflammation is not a protective response and can damage healthy tissues over time [9, 10]. In addition to these two main types of inflammation, there are also other subtypes of inflammation, including: [11, 12, and 13]. Systemic inflammation: This type of inflammation affects the whole body and is associated with a range of chronic diseases, including obesity, metabolic syndrome, and cardiovascular disease. Neuroinflammation: This type of inflammation occurs in the brain and is associated with a range of neurological disorders, including Alzheimer's disease, Parkinson's disease, and multiple sclerosis. Allergic inflammation: This type of inflammation occurs in response to allergens, such as pollen, dust mites, or certain foods, and is associated with allergic reactions, including asthma, hay fever, and hives. Autoimmune inflammation: This type of inflammation occurs when the immune system mistakenly attacks healthy tissues in the body, leading to a range of autoimmune disorders, including rheumatoid arthritis, lupus, and multiple sclerosis. These are problems can be treat by ayurvedic treatment. Scientific evidence suggests that black garlic, which is rich in S-allylcystein, as well as polyphenols from cat's claw (*Uncaria tomentosa*), devil's claw (*Harpagophytum procumbens*), camu-camu (Myrciaria dubia), and blackcurrant (Ribes nigrum), and citrus fruit extracts containing hesperidin, exhibit similar or greater effects than other well-studied extracts such as tea and cocoa. The combination of these extracts has the potential to produce synergistic effects with greater biological relevance at lower doses. In accordance with the polyherbal synergistic hypothesis, we have selected three natural plants, namely *Parmotrema reticulatum, Curcuma caesia*, and *T. patula*, to investigate their anti-inflammatory properties. These plants represent promising alternatives and offer a novel concept for the development of new drugs that can be used to treat topical inflammatory diseases [14-20].

MATERIAL AND METHODS

Plant materials were collected local market from Bhopal region of Madhya Pradesh., India. Identified and authenticated was done by plants materials are verified by Pharmacognosist, Dr. Sandeep Kumar Singh, at the Central Ayurvedic Research Institute in Jhansi, Uttar Pradesh, with accession numbers CARI/H/13302021, CARI/H/13302022, CARI/H/13302023 Botanical Survey of India, Central Regional Centre, Praygraj, U.P.

2.2 Extraction of plant material

Plant material of *Permoterma reticulatum, Curcuma caesia* and *T. patula* were extracted separately by using cold maceration method; plant samples were collected, washed, rinsed and dried properly. Powder form of plant sample was extracted with hydroalcohalic solvent (30:70) and allows standing for 4-5 days each. The extract was filtered using filter paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. Extract was transferred to beaker and evaporated; excessive moisture was removed and extract was collected in air tight container. Qualitative analysis of extracts of different solvents was carried out to find out the presence of various phytoconstituents [21 Extraction yield of all extracts were calculated using the following equation below:

$$\textbf{Percentage Yield} = \frac{\textbf{Actual yield}}{\textbf{Theoretical yield}} \times 100$$

Qualitative phytochemical estimation of extracts

Phytochemical Estimation of *Permoterma reticulatum, Curcuma caesia* and *T. patula* extracts were determined using standard techniques such as alkaloids, flavonoids, tannins, phenol, saponins and glycosides etc.

Preliminary in-vitro anti-inflammatory assays

Protein denaturation assay

In-vitro anti-inflammatory assays of *Permoterma reticulatum, Curcuma caesia* and *T. patula* were

performed according to the protocol described earlier with some modification. Reaction mixture was prepared by mixing varying concentrations of different extracts viz 100, 250 and 500µg/ml to 5% bovine albumin albumin in separate test tubes. Standard drug, Acetyl salicylic acid with standard drug was taken as positive control. Experiment was repeated three times and the percentage inhibition of protein denaturation exhibited by each extract was calculated as under:

$$\textbf{Percentage inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{\text{Absorbance of control}} \text{ x } 100$$

RESULT AND DISCUSSION

Extraction of Selected plant materials

Extraction of Selected *Permoterma reticulatum, Curcuma caesia* and *T. patula* were prepared by using leaves (10.66 kg), different solvent water, pet ether, chloroform and methanol. The yield of extract was found to be 8.3%, 3.1%, 8.3% and 7.6 % respectively. The results of yield of extract indicated that highest bioactive components present in water, chloroform and methanol extract. These extract may have potential therapeutic activities [20-22].

Table 2: Extractive values obtained from *Permoterma reticulatum, Curcuma caesia* and *T. patula*

S.N.	Solvent	Color of extract	% yield w/w
1	*Permoterma reticulatum*	Dark Bluish	8.3
2	*Curcuma caesia*	Blackish	3.1
3	*T. patula*	Dark Bluish	7.6

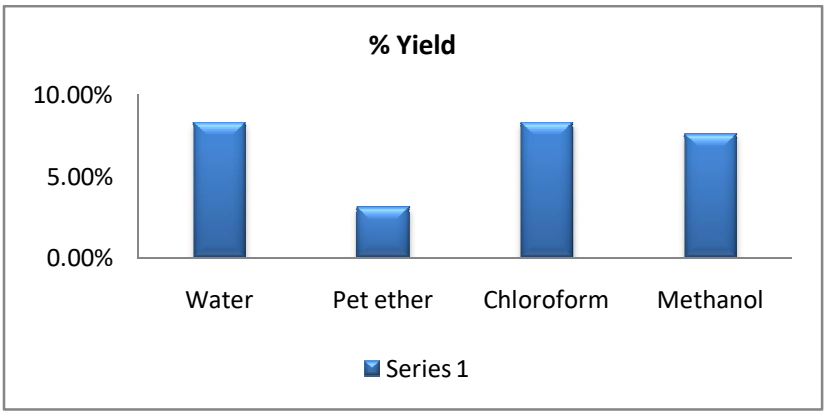

Figure 5: Graph of Extractive values obtained from *Permoterma reticulatum, Curcuma caesia* and *T. patula* extract

Phytochemical screening test

According to research, PRHE has the best potential for bioactivity of any extract when it comes to glycosides, alkaloids, glycosides, phenolic compounds, tannins, saponins, flavonoids, and proteins. Hence, PRHE chose us for additional research. Results are shown in below Table 1.

Table 1: Results of phytochemical screening test of Hydroalcohalic Extract

S.N.	Constituents	*P.reticulatum*	*C. caesia*	*T. patula*
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Phenols	+	+	+
4	Tannins	+	+	+
5	Saponins	+	+	+
6	Steroids & terpenoids	+	+	+
7	Glycosides	-	-	-
8	Carbohydrates	+	+	+
9	Anthraquinones	+	+	+

(+) indicates presence of phytochemicals; (-) indicates absence of phytochemicals

Phytochemical constituents of *Permoterma reticulatum, Curcuma caesia* and *T. patula* extract were determined and found to be highest bioactive constituents present in water, methanol and ethanol

respectively, due to highest polar nature of solvent. It indicated that these solvent having highest potential for the pharmacological action [23-24].

FTIR spectra of *Permoterma reticulatum, Curcuma caesia* and *T. patula*

The leave extracts of *Permoterma reticulatum, Curcuma caesia* and *T. patula* indicated the presence of active biological compounds such as flavonoids, proteins, phenols, alkaloids and glycosides. In FTIR analysis, different peaks of functional gropus of bioactive compounds were identified. The finding results were revealed the presence of flavonoids, phenols, glycosides and alkaloids, respectively. The results are shown in Table 8 and Fig. 10.

Table 8: FTIR peaks of ethanol extract of *Permoterma reticulatum, Curcuma caesia* and *T. patula*

S.N.	Sample	Rf Value	Colour	Resolution
1	A	0.06	Yellow	Good
2	B	0.72	Green	Excellent
3	C	0.12	Brown	Best
4	D	0.78	Dark green	Excellent

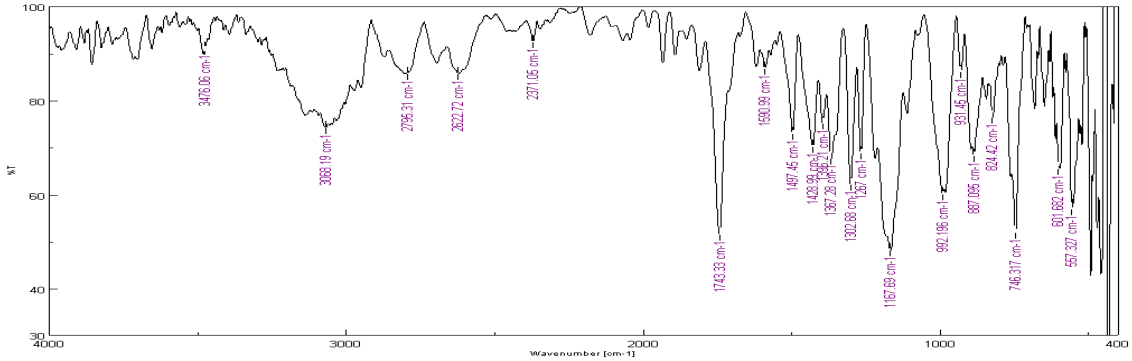

Figure: FTIR spectra of *Permoterma reticulatum, Curcuma caesia* and *T. patula* extracts

***In-vitro* anti-inflammatory effects of *Permoterma reticulatum, Curcuma caesia* and *T. patula* extracts**

Effect of *Permoterma reticulatum, Curcuma caesia* and *T. patula* extracts on protein denaturation. Among the three tested extracts of *Permoterma reticulatum, Curcuma caesia* and *T. patula*, it was found that *Curcuma caesia* hydroalcohalic extract was most potent in suppressing protein denaturation with an inhibition percentage of 79.8, 59.1 followed by 36.2**%** at the concentration of 500 µg/ml respectively.

Table 9: Percentage inhibition of protein denaturation exhibited by various extracts of *Permoterma reticulatum, Curcuma caesia* and *T. patula*

Group	% inhibition
Normal group	65.2±.23
Standard group	100.11±.04
Hydroalcohalic extract of PR (500 µg/ml)	59.1±.12
Hydroalcohalic extract of CC (500 µg/ml)	84.8±.02
Hydroalcohalic extract of TP (500 µg/ml)	36.2±.05

IC50 value was 106±26, Diclofenac Sodium 108.06 ± 4.60 µg/mL Positive control 53.18 ± 0.28a

The shade dried plant material was converted into moderately coarse powder. Ash content value of *Permoterma reticulatum, Curcuma caesia* and *T. patula* was FTIR bands were observed under. The various extract obtained after the extraction were subjected to phytochemical screening for determination of various class of pharmaceutical constituents present in *camellia sinensis*. Alkaloids, Flavonoids, Phenols, Tannins, Saponins, Steroids & terpenoids, Carbohydrates were found positive in camellia sinensis [20-24].

CONCLUSION

Our research work were investigated phytochemical and FTIR spectral results indicated that *Permoterma reticulatum, Curcuma caesia* and *T. patula* having potential Alkaloids, Flavonoids, Phenols, Tannins, Saponins bioactive etc compounds are present. The anti inflammatory activity of *Permoterma reticulatum,*

Curcuma caesia and *T. patula* extracts was investigated and determined by using *In-vitro* percentage inhibition of protein denaturation method. The evaluation results of five tested different extracts of *Permoterma reticulatum, Curcuma caesia* and *T. patula* on percentage inhibition of protein denaturation method indicated that *Permoterma reticulatum, Curcuma caesia* and *T. patula* hydroalcohalic extract was exhibited potent protein suppressing denaturation property close to standard drug. The highest percentage inhibition of protein denaturation was found to be 84.8, 59.1 followed by 36.2 at the concentration 500 µg/ml respectively. Results of percentage inhibition of protein denaturation represented that *Permoterma reticulatum, Curcuma caesia* and *T. patula* having important source of bioactive components and need to identity the quality and purity of the plant material in future studies.

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Conflict of interest
The Authors declare no conflict of interest

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