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Evaluation of Anti-Oxidant Activity of Different Extracts of *Boerhaavia Diffusa*: An In-Vitro Approach

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ABSTRACT:

AIM- The aim of the present investigation is to study the anti-oxidant activity of various extracts of Punarnava root. **MATERIAL METHODS**- Dried plant material was powdered and it was packed and sealed in pouch made from cotton fabric and placed in thimble. 0.5 ml of Folin-Ciocalteau reagent and 5 ml of distilled water were combined with a volume of 1 ml from the extract solution, which had been prepared at concentrations of either 1 mg/ml or 0.1 mg/ml. In the determination of total flavonoid content, the aluminum chloride colorimetric assay was employed. The assessment of free radical scavenging activity in bioactive extracts was conducted using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. In the ABTS assay, a 7 mM solution of ABTS was prepared, and its radical cation (ABTS+•) was generated by reacting it with 2.45 mM potassium persulfate, followed by a 12–16 hour incubation in darkness. **RESULTS**- The extracts of BF were tested for presence and/or absence various phytochemicals like alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids etc. The content of phenolic compounds was determined from the regression equation of a calibration curve (y=0.011x±0.011) expressed as mg of the both Equivalents (GAE) per mg of extract. The content of flavonoids was determined from the regression equation of a calibration curve (y = 0.1408x + 0.0822, R2 = 0.9983) expressed as mg of Rutin Equivalents (RE) per gram of extract. **CONCLUSION**- The research on the pharmacological evaluation of indigenous medicinal plants for neuropathic pain represents a significant and promising endeavour that holds the potential to address the limitations of current therapeutic approaches.

Keywords: Anti-oxidant Approach, Various Extracts, Boerhaavia diffusa, In-vitro Approach, Polar & Non Polar Extracts

1. Introduction

Indigenous medicinal plants have been a source of healing for various ailments for centuries, offering a rich repository of traditional knowledge. Many indigenous communities have developed a profound understanding of the therapeutic properties of local flora, passing down this knowledge through generations. The study builds upon this wealth of ethnobotanical wisdom, recognizing the potential of indigenous medicinal plants to offer novel solutions to the complex problem of neuropathic pain. Additionally, the escalating interest in natural products and complementary medicine has fueled a global exploration of traditional remedies. Indigenous medicinal plants, with their diverse chemical profiles, present an intriguing avenue for drug discovery. The identification and isolation of bioactive compounds from these plants may unlock new possibilities for neuropathic pain management [1]. The urgency of this research is further underscored by the need for safer and more tolerable treatments. Conventional medications often carry the risk of adverse effects, such as sedation and addiction, prompting a quest for alternative therapies with a more favourable safety profile. By delving into the pharmacological properties of select indigenous plants, this study seeks to identify compounds that not only alleviate neuropathic pain but also minimize the risk of undesirable side effects [2]. The background to this study is grounded in the unmet clinical needs associated with pain management, the wealth of traditional knowledge surrounding indigenous medicinal plants, and the global interest in exploring natural remedies. The research aims to bridge the gap between ancient healing practices and contemporary medicine, offering a promising avenue for the development of innovative and effective treatments for pain management through anti-oxidant activity.

2. Materials and Methods

Plant Material and Phytoconstituent

Punarnava roots were purchased from Shriji Ayurvedic Centre at Boriwali, Mumbai. All the plant materials were sent for authentication to Agharkar Research Institute, Pune, Maharashtra. Boerhaavia diffusa was purchased from HIMEDIA.

Processing of Crude Plant Material

Plant materials were authenticated, subsequently pulverized, and stored in hermetically sealed containers to ensure preservation. The pulverized plant materials were then employed for the extraction process.

Preparation of Plant Extracts

Dried plant material was powdered and it was packed and sealed in pouch made from cotton fabric and placed in thimble. The solvent was placed in distillation flask. The vapours of solvent were made to pass through the thimble containing plant material and were liquefied in the condenser [3].

When the liquid reaches the overflow level in thimble, siphon aspirates the solution, which flows back to distillation flask, carrying the extracted solutes into bulk solvent. The extraction process was carried out for period of 30 hrs.

Literature search was carried out for selection of solvents, and it was revealed that polar solvents like water, ethanol, methanol etc. were used for extraction of bioactive phytoconstituents. The powdered plant material: solvent ratio was 1:5. The percentage yield of the extracts was calculated [4].

Physicochemical evaluation of plant extracts

The plant extracts were subjected to a comprehensive assessment of their physicochemical attributes, encompassing parameters such as color, consistency, solubility, pH, and percent yield (% w/w). This thorough evaluation aimed to provide a comprehensive understanding of the extracts' physical and chemical characteristics.

Phytochemical screening of plant extracts

A one-gram aliquot of each plant extract was dissolved in 100 ml of methanol, resulting in a stock solution with a concentration of 1% (v/v). Subsequently, the filtrate obtained through this process was employed for the identification of diverse phytochemical constituents using a series of specific tests [4, 5].

Phenolic Quantification Assay is based on Folin-Ciocalteu method.

In the experiment, 0.5 ml of Folin-Ciocalteau reagent and 5 ml of distilled water were combined with a volume of 1 ml from the extract solution, which had been prepared at concentrations of either 1 mg/ml or 0.1 mg/ml. The final amalgamation underwent a 10-minute incubation period at room temperature. The final volume was then increased to 10 ml by adding 1.5 ml of anhydrous sodium carbonate solution with a 10% w/v concentration. An essential component of the Folin-Ciocalteu method, which is used to quantify the total phenolic content in the test materials, is this standardized protocol.

The quantification of total phenolic content was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of the plant extract. Each test was performed three times, and the findings are reported as mean plus or minus standard deviation. This thorough technique ensures that the experiment's results are precise and trustworthy [6].

Determination of Total flavonoid content (TFC) of extracts

In the determination of total flavonoid content, the aluminum chloride colorimetric assay was employed, following a protocol detailed by Bhaigyabati, Bag, and Grihanjali Devi in 2015. In this method, a 1 ml aliquot of each extract or a standard rutin solution at varying concentrations (50, 100, 150, 200, 250, and 300 mg/l) was mixed with 4 ml of distilled water. Subsequently, 0.3 ml of a 5% $NaNO_2$ solution was added, and after a 6-minute incubation period, 0.3 ml of a 10% $AlCl_3$ solution was introduced. Following an additional 6-minute incubation, 2 ml of a 1M NaOH solution was incorporated into the mixture. To adjust the final volume to 10 ml, water was added, and the resulting mixture was thoroughly mixed and allowed to stand for an additional 15 minutes. The absorbance of the pink color developed in the solution was then measured at a wavelength of 510 nm. This comprehensive approach allows for the quantification of total flavonoid content, utilizing the absorbance at 510 nm as an indicator, with rutin serving as a standard for calibration [7].

In-vitro Antioxidant Activity

DPPH radical scavenging activity

The assessment of free radical scavenging activity in bioactive extracts was conducted using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, following the approach outlined by Blois in 1958. A 0.1 mM solution of DPPH in methanol was prepared for the assay. Subsequently, 1 ml of the extract solution at varying concentrations was added to 3 ml of the 0.1 mM DPPH solution. The reduction in absorbance was measured at 517 nm after a 30-minute incubation period. The percentage scavenging activity was calculated using the formula $[(A0–A1)/A0] \times 100$, where A0 represents the absorbance of the control (DPPH solution without the test compound), and A1 is the absorbance of the extract or standard. A blank was determined as the absorbance of the control reaction containing all reagents except the test compound. To

quantify the antioxidant activity, both the percentage scavenging activity and the IC50 value (the concentration at which 50% of DPPH radicals are scavenged) were calculated for various concentrations of the extracts. Ascorbic acid was employed as a standard antioxidant for comparative purposes. This methodology provides a comprehensive assessment of the free radical scavenging potential of bioactive extracts, enabling a comparison with a known standard antioxidant [8].

ABTS assay

In the ABTS assay, a 7 mM solution of ABTS was prepared, and its radical cation (ABTS+•) was generated by reacting it with 2.45 mM potassium persulfate, followed by a 12–16 hour incubation in darkness. The resulting stable ABTS+• solution, lasting for 2 days, was diluted with a 2 mM phosphate buffer at pH 7.4 to achieve an absorbance of 0.8 ± 0.014 at 734 nm. Extract solutions were then mixed with the ABTS+• solution, and absorbance readings were taken at room temperature after 1 minute. A phosphate buffer solution served as the blank. The radical-scavenging activity of the samples was expressed based on their ability to reduce the absorbance of the ABTS radical cation, with lower absorbance values indicating higher antioxidant potential. This method provides a quantitative measure of the antioxidant capacity of the tested extracts in relation to the ABTS radical cation, allowing for a comprehensive assessment of their radical-scavenging activity [9].

% Radical Scavenging = [(A (control)–A (test))/ A (control)] X 100%,

Ascorbate-iron induced lipid peroxidation assay

In this experiment, 10 mg of bovine brain extract was combined with 2 ml of phosphate buffer at pH 7.4. The mixture was then sonicated in an ice bath until a milky-like suspension was achieved, containing phospholipid liposomes. Subsequently, 0.2 ml of the liposome suspension was mixed with 0.1 ml of 1 mM FeCl3 and the bovine brain extract. The total volume was adjusted to 0.5 ml using phosphate buffer.

The peroxidation process was initiated by adding 0.1 ml of 1 mM ascorbate to the mixture. Following the addition of ascorbate, the entire mixture was incubated at 37°C for a duration of 60 minutes. This experimental setup is designed to induce lipid peroxidation, likely involving the generation of reactive oxygen species due to the interaction of ascorbate with FeCl3 in the presence of liposomes. The incubation at 37°C simulates physiological conditions and allows for the assessment of the potential protective or inhibitory effects of the bovine brain extract on lipid peroxidation over the specified time frame [9].

3. Results and Discussion

Organoleptic Properties

By employing a combination of organoleptic, physical, and physicochemical parameters in the standardization process, researchers aim to establish a set of criteria that can be used to consistently evaluate and authenticate the plant material.

TABLE NO. 1: Organoleptic Evaluation of The Plant Materials

Organoleptic characters	**PUNARNAVA**
Colour	White
Odour	Slightly piquant liquor aroma.
Taste	Bitter and sweet taste
Shape	Ovate-oblong
Texture	Hairy-textured under-side

Physicochemical analysis:

The variations in extractive values with different solvents emphasize the importance of selecting an appropriate solvent based on the desired phytochemical profile. Analyzing water and alcohol-soluble extractive values provides insights into the diversity of compounds present in the herbal material, aiding researchers, herbalists, and pharmacologists in characterizing and standardizing herbal medicines for optimal therapeutic outcomes.

TABLE NO. 2: Physicochemical Analysis of Plant Material

Plant Name	Total ash	Acid insoluble ash	Loss on drying	Water soluble extractive	Alcohol-soluble extractive
PUNARNAVA	8.32	1.04	5.43	24.21	14.21

Phytochemical studies of plant extracts

The extracts of BF were tested for presence and/or absence various phytochemicals like alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids etc. The results of preliminary phytochemical studies are shown in Table respectively.

TABLE NO. 3: Preliminary Phytochemical Screening of Extracts

S.No	Test	BD-MET	BD-ETH	BD-EtAC	BD-WA	BD-HA
1	Alkaloids	-	-	-	-	-
2	Carbohydrates	+	+	+	+	+
3	Glycosides	+	-	-	-	-
4	Saponins	+	+	+	+	+
5	Phenolic compounds	+	+	+	+	+
6	Proteins and amino	-	-	-	-	-
7	Flavonoids	+	+	+	+	+
8	Sterols and terpenoids	+	-	+	-	+

In vitro evaluation of antioxidant activity

Determination of total of phenolic content (TPC) of plant extracts

The calibration curve for standard punarnava was plotted. The dilutions were prepared in the optimised range of 10 to 100 µg/ml, by plotting concentration vs. absorbance. The content of phenolic compounds was determined from the regression equation of a calibration curve (y=0.011x±0.011) expressed as mg of the both Equivalents (GAE) per mg of extract [10].

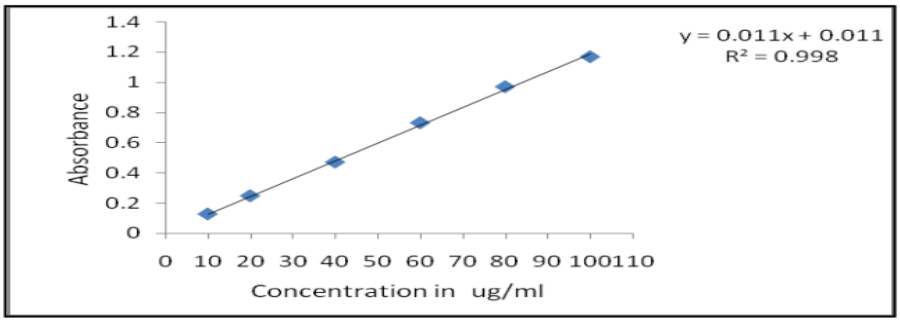

FIGURE 1: Calibration Curve for Punarnava

Determination of total of flavonoid content (TFC) of plant extracts

The calibration curve for standard rutin was established in the optimised range from 100µg/ml to 600 µg/ml by plotting concentration vs. absorbance. The content of flavonoids was determined from the regression equation of a calibration curve (y = 0.1408x + 0.0822, R2 = 0.9983) expressed as mg of Rutin Equivalents (RE) per gram of extract [10].

TABLE NO. 4: Total Phenolic Content

Extract	**TPC in 1 mg of extract (std)**
BD-MET	68.09±0.76
BD-ET	56.66 ± 0.58
BD-HA	72.27 ±0.28
BD-EtAC	21.87 ± 0.67
BD-WA	59.57 ± 0.43
BD-MET	29.18±0.24
BD-ET	53.00±0.37
BD-EtAC	20.37±0.78
BD-WA	47.09±0.95
BD-HA (40: 60)	28.21±0.71
BD-HA (90: 10)	33.97±0.49

Among the BD extracts, BD-HA displays two entries, suggesting potential variations in extraction conditions or formulations. The first instance indicates a relatively high TPC of 72.27±0.28 mg GAE/mg, while the second instance, with different ratios (40:60 and 90:10), exhibits TPC values of 28.21±0.71 mg GAE/mg and 33.97±0.49 mg GAE/mg, respectively. These variations may signify the impact of differing ratios on the phenolic content.BD-MET demonstrates a noteworthy TPC of 68.09±0.76 mg GAE/mg, indicating a substantial presence of phenolic compounds. BD-ET follows closely with a TPC of 56.66±0.58 mg GAE/mg, displaying a considerable but slightly lower phenolic content than BD-MET. BD-WA also exhibits a substantial TPC of 59.57±0.43 mg GAE/mg.BD-EtAC shows a comparatively lower TPC of 21.87±0.67 mg GAE/mg, suggesting a reduced phenolic content in this particular extract. Researchers and practitioners can leverage this data to understand the antioxidant potential of these extracts, guiding their applications in diverse fields such as pharmaceuticals or functional foods [11].

DPPH radical scavenging activity

DPPH radical is a commonly used substrate for rapid evaluation of antioxidant activity because of its stability in the radical form and simplicity of assay. DPPH is nonspecific radical and scavenging of DPPH radical gives reliable information about the antioxidant ability of the extracts.

TABLE NO. 5: DPPH Scavenging Activity of All Plant Extracts

Extract	**IC50 value (µg/ml)**
BD-MET	69.66 ±0.05
BD-ET	69.16 ±0.12
BD-HA	147.02 ± 0.07
BD-EtAC	38.53 ±0.04
BD-MET	468.37±0.06
BD-ET	215.63±0.07
BD-EtAC	396.77±0.01

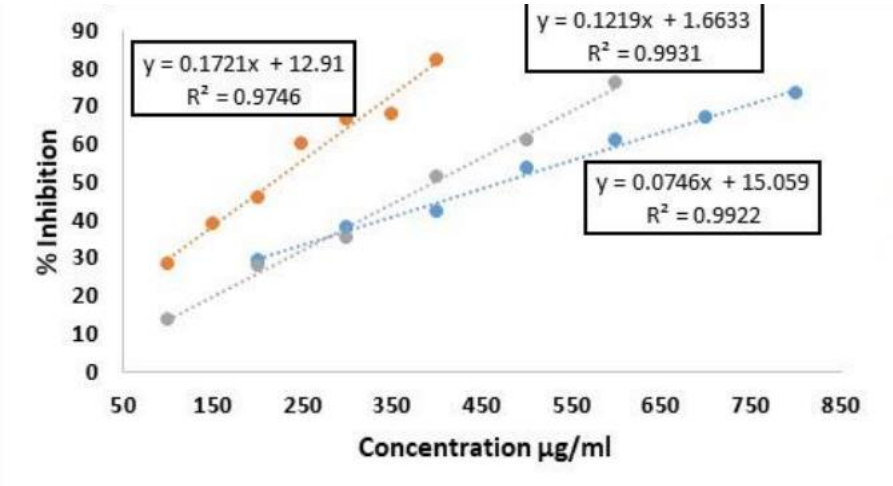

FIGURE 2: DPPH Scavenging Activity of BD Extracts

ABTS assay

Like DPPH radical, ABTS radical is also not generated in vivo. It is nonspecific and in vitro method for determining the antioxidant activity of a substance.

BD-ET showed highest IC50 amongst all the plant extracts of 31.45±0.27. BD-EtAC showed highest inhibition with IC50 value of 35.74±0.16 as compared to other GGR extracts; i.e., GGR-MET with IC50 131.47±9.2 and BD-ET with IC50 111.36±1.4. The IC50value of BD-ET was 21.34±0.27 [12].

TABLE NO. 6: ABTS assay of All Plant Extracts

Extract	IC50µg/ml
BD-MET	152.27±8.3
BD-ET	101.36±2.9
BD-EtAC	32.74±0.23
BD-ET	25.25±0.65
BME	522.29±2.2
Ascorbic acid	6.54±0.11

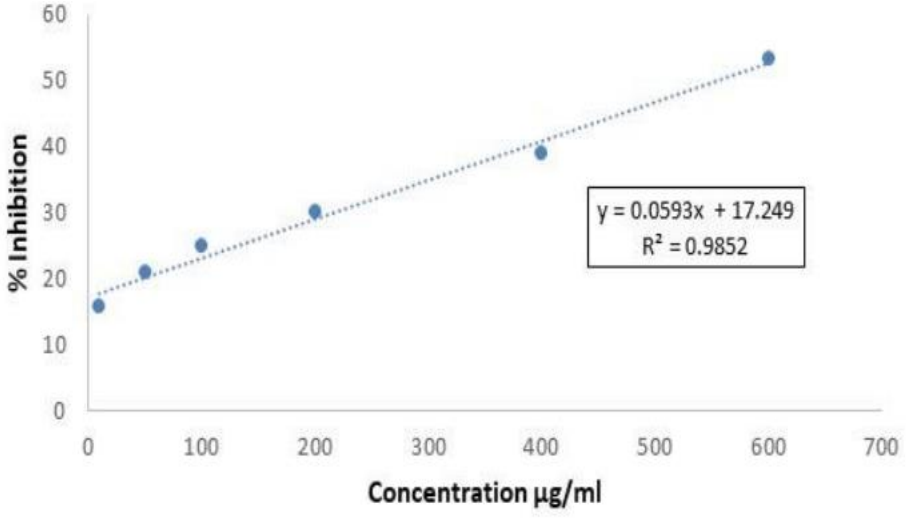

FIGURE 3: ABTS Scavenging Activity of BD

Acerbate-iron induced lipid peroxidation assay

The process of lipid peroxidation, involving the breakage of lipids and the formation of reactive compounds, is well-established to induce changes in the permeability and fluidity of the membrane lipid bilayer.

TABLE NO. 7: Lipid Peroxidation Activity of Plant Extracts and Standard.

Extract	**IC50 value (µg/ml)**
BD-MET	876.11±0.07
BD-ET	221.42±0.05
BD-EtAC	123.59±0.06
Standard-BHT	54.12±0.08

The table presents IC50 values (half-maximal inhibitory concentration) for different extracts, namely BD-MET, BD-ET, and BD-EtAC, along with a standard reference, BHT (Butylated Hydroxytoluene), measured in µg/ml. The IC50 values signify the concentration at which a substance inhibits a specific biological or biochemical function by 50%.

For BD-MET, the IC50 value is reported as 876.11±0.07 µg/ml, for BD-ET it is 221.42±0.05 µg/ml, and for BD-EtAC it is 123.59±0.06 µg/ml. Lower IC50 values indicate a higher potency of the extract in inhibiting the targeted function. The standard BHT exhibits an IC50 value of 54.12±0.08 µg/ml, suggesting its higher potency compared to the BD extracts [13].

The data suggests that BD-EtAC has the lowest IC50 value among the BD extracts, indicating its superior inhibitory activity against the specified function. The comparison with the standard BHT underscores the potential inhibitory efficacy of the BD extracts, with BHT showing a higher potency [14, 15].

These IC50 values provide quantitative insights into the relative inhibitory potency of each BD extract, aiding in the evaluation of their potential pharmacological or therapeutic applications [16]. Researchers and practitioners can utilize this information to understand the efficacy of these extracts in specific contexts, contributing to the exploration of their biological activities [17, 18].

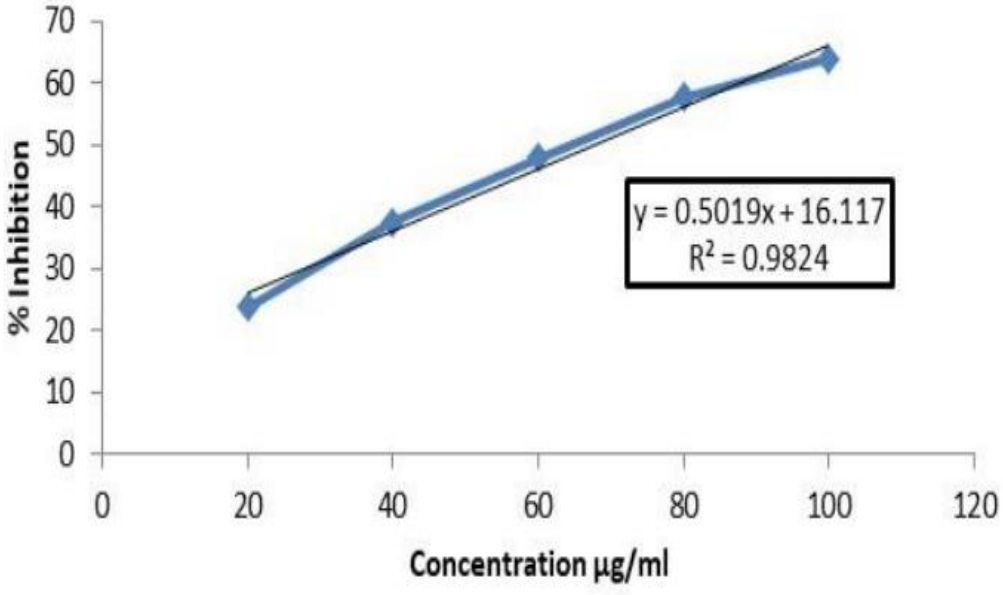

FIGURE 4: Lipid Peroxidation Activity of BD

4. Conclusion

The research on the pharmacological evaluation of indigenous medicinal plants for neuropathic pain represents a significant and promising endeavor that holds the potential to address the limitations of current therapeutic approaches. The multifaceted nature of neuropathic pain, coupled with the inadequacies of existing treatments, underscores the importance of exploring alternative interventions, and this study aims to contribute valuable insights to this complex field. The systematic review and selection of indigenous medicinal plants based on traditional knowledge and ethnobotanical relevance lay the foundation for a comprehensive exploration of their pharmacological potential. By bridging the gap between ancient healing practices and modern scientific methodologies, this research integrates the rich tapestry of traditional wisdom with rigorous pharmacological evaluation.

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