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Pharmacognostical Evaluation and *In vitro* Antioxidant and Anti-inflammatory Activity of *Exacum bicolor* Roxb.

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Abstract

The primary goal of this research was to investigate the morphological and microscopical characteristics of various parts of *Exacum bicolor* Roxb, as well as the antioxidant and anti-inflammatory activity of different extracts. The morphology and microscopy of leaves, stem, flower and root were studied, along with powder characteristics of the whole plant. The whole plant was powdered and extracted using various solvents such as chloroform, acetone methanol and water. Antioxidant activity of various extracts of the plant was determined by DPPH method and nitric oxide scavenging assay method. The protein denaturation and membrane stabilization methods were used to assess anti-inflammatory activity. Microscopic examination revealed that it is a dorsiventral leaf with covering trichomes. The histology of roots showed the phloem encircles the xylem, and the xylem with distinct vessels and lignified xylem parenchyma. The powder microscopical characteristics are stomata, epidermal cells, trichomes, xylem vessels, xylem fibers, *etc*. These features of the plant can be used as a tool for the development of a monograph. The chloroform extract showed a better antioxidant activity compared with other extracts, while methanolic extract showed good anti-inflammatory activity in comparison between the extracts.

Keywords: *Exacum bicolor* Roxb., morphology, microscopy, antioxidant, anti-inflammatory.

1. Introduction

Plant anatomy is still a valuable tool for resolving perplexing issues in botanical research at the national level. Anyone who works with plants has to understand plant anatomy. When publishing experimental data, many researchers thoroughly neglect the need to understand anatomy (Cutler et al., 2008). Plant anatomy is traditionally considered the microscopic study of plant tissues and cells, and the invention of light, and electron microscopes has greatly aided our understanding of the structure (Crang *et al*., 2018).

Exacum bicolor Roxb. (E.bicolor) is an angiosperm in the *Gentianaceae* family which is a family of flowering plants with 84 genera and 1688 species (Rajisha and Jennifer, 2020a).*E.bicolor Roxb.* is an erect herb that grows in the plains from July to November and in the high mountains from July to January. The whole plant has medicinal properties such as tonic, antipyretic, and promoting appetite. This plant can also be used to produce dye. People use this as traditional medicine in case of elevated glucose levels and skin problems due to its bitter taste. Traditional Kerala practitioners recommend washing the eyes with a decoction of the entire plant. However, due to its limited distribution and accessibility concerns, it is not commonly used. (Sreelatha *et al*., 2007; Rajisha and Jennifer, 2020b).

The presence of specific bioactive components in certain amounts is the main reason for the therapeutic activity of plants. Such issues are often resolved by fixing exact specifications about the pharmacognostic characteristics of plant drugs. Pharmacognostic evaluations drive support in the validation of a plant and guarantee plant drug efficacy and safety.

Chemical composition information on the genus Exacum is limited. Exacum species have enormous pharmacological potential, which has been demonstrated by their widespread use in conventional medicine and has been backed by ethnobotanical research and investigations into particular biological activity. Due to overexploitation, many Exacum species are endemic and frequently in danger of extinction. Micropropagation can assist defend those species and help introduce them to commercial floriculture. (Skrzypczak-Pietraszek E, 2015).

Although the plant has a traditional medicinal value, data on its quality control profile is still unavailable. This study aims to develop a scientific standardization monograph for *E.bicolor* Roxb. and the antioxidant and anti-inflammatory activity of the different plant extracts. This information helps to identify adulterants and to ensure the quality of herbs.

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2. Methodology

2.1. Plant material

In the month of August-November, plant *E.bicolor* Roxb. was collected from regions of Kerala (Kannur and Kasaragod). It was identified and authenticated taxonomically by Dr. K. Gopal Krishna Bhat, Professor of Botany(Rtd.). A specimen voucher (17PH001R) was preserved in the Department of Pharmacognosy of Nitte Gulabi Shetty Institute of Pharmaceutical Sciences, Mangalore, Karnataka, India.The whole plant was collected, cleaned, and further utilized for the morphological and microscopical studies of leaf, stem, and root. The remaining plants were shade-dried, powdered and used for powder microscopic analysis.

2.2. Extraction of plant material

Prior to Soxhlet extraction, the powder was defatted with petroleum ether, weighed and then placed in the Soxhlet apparatus. The extraction was performed using the method developed by KanikaDulta*et.al.* with slight modification. The Soxhlet extraction was carried out with chloroform, acetone, and methanol by successive solvent Soxhlet extraction (KanikaDulta*et.al,* 2021). The marc, after methanol extraction, was mixed with water and boiled in a water bath. The extracts were filtered, the solvents were distilled, and the pure extract was obtained.

2.3. Morphological studies

The morphological evaluation of the whole plant of *E.bicolor* Roxb. was carried out. Various organoleptic features of different parts of plant like leaves, stem, flowers and fruits (colour, taste, odour, shape, size) were evaluated.

2.4. Microscopical studies

Suitable sections of plant part (root, stem and root) were taken for anatomical evaluation. By using a microtome, transverse sections of leaf, stem and root were taken. The proper sections with a thickness of 10-15 µm were selected and warmed with chloral hydrate solution, which acts as a clearing agent. These clear sections were further stained with phloroglucinol and with 1-2 drops of conc. HCl. After two minutes, these sections were transferred to glass slides and added a drop of glycerine. The transverse sections were enclosed with the help ofa cover slip and observed under a microscope (Biovis IP2000 digital microscope) from both sides (Kandalwal, 2005; Ghaid J.Al-Rabadi, 2014).

2.5. Powder microscopical studies

The whole plant was collected and washed thoroughly with water to remove soil and other adhesive material for powder analysis. The plant was dried under shade, and this was followed by powdering the whole plant; the obtained powder was passed through sieve no. 60. Minimal amount of the powder was boiled with chloral hydrate followed by staining it with conc. HCl (1:1) and phloroglucinol solution to find various kinds of tissues proving authenticity. To find calcium oxalate crystals, one more sample was mounted in water; and to observe starch grains, one sample was mounted in an iodine solution. (Kandalwal,2005;Haeborne,1973).

2.6. Determination of leaf constant

Fully developed leaves were used to quantify the stomatal density and stomatal index. The lower epidermis was pulled off from the middle section of the leaf. In total, 5 distinct leaves were used to calculate the stomatal density. Along with stomatal density, the epidermal layer is also used to determine the stomatal index. The stomatal index was calculated by using the following formula

$SI = S / E + S \times 100$

Where,

SI -Stomatal index

S-Number of stomata cells per unit area

E -number of epidermal cells per unit area.

The vein islet number as well as vein termination number are determined by using the leaves lamina (Paul V*et.al.*2017).

2.7. In vitro biological assays

2.7.1. Antioxidant activity

2.7.1.1. DPPH scavenging activity

A chemical 2, 2-diphenyl-1-picrylhydrazyl (DPPH)since spare electron is delocalized over a entire molecule, preventing dimerization; it is characterized as a persistent free radical as is a case with most other free radicals. The deep violet color is caused by electron delocalization, which is characterized by a 517 nm absorption band in ethanol (Alam *et. al*.,2013).

1 ml of 0.3 mM alcoholic solution of DPPH was added to 2.5 ml of the samples with varying concentrations (6.25-100µg/ml) of *E.bicolor* Roxb. and standard ascorbic acid. Ascorbic acid is a very good antioxidant; it protects the cellular component from free radical damage. After 30 minutes of reaction at room temperature in the dark, the absorbance was measured at 517 nm(Dahiru Daniel and Thagriki Dluya, 2016). The percentage of DPPH radical inhibition was estimated by comparing the test results to those of the control group using the formula below:

Percentage of inhibition = $[(A_0 – A_1) / A_0] \times 100$

Where A_0 = Absorbance of the control,A_1 = Absorbance of the sample/ standard(Muntana and Prasong, 2010).

2.7.1.2. Nitric Oxide Scavenging Assay

0.5 mL phosphate buffer saline (pH 7.4) was used to dilute 2 mL sodium nitroprusside (10 mM) and was treated with 0.5 mL sample at different concentrations (6.25-100µg/ml) and incubated for 2h 30min. 0.5 mL of the reaction mixture was pipetted out after incubation.1 mL of sulfanilic acid was added to it. Further, it was allowed to stand for another 5 minutes to complete diazotization. After that, 1ml of 1-naphthylamine was added, stirred, and set aside for 30 minutes. In diffused light, a pink chromophore was generated. A control was also made with the same solutions without the sample drug or standard drug. The solution's absorbance was measured at 546nm in comparison to a blank solution. Calculation of percentage inhibition of nitric radical was carried out with the formula:

Percentage of inhibition = $[(A_0 – A_1) / A_0] \times 100$

Where A_0 = Absorbance of the control, A_1 = Absorbance of the plant extract/ standard (Marcocci L *et al*., 1994).

2.7.2. Anti-inflammatory activity of whole plant extracts

2.7.2.1. Inhibition of protein denaturation method

The reaction mixture (0.5 ml) comprised of 0.05ml plant extracts (acetone, chloroform, aqueous and methanol) and 0.45 ml of bovine serum albumin (5% aqueous solution) and pH was adjusted to 6.3 with the help of 1N HCl. For 20 min samples were incubated at 37^0C followed by heating it for 3min at 57^0C. Diclofenac was used as a standard drug (6.25-100µg/ml). Diclofenac is a nonsteroidal anti-inflammatory drug, and it inhibits COX-2 enzyme. After cooling, the samples, 2.5ml phosphate buffer saline (pH 6.3) were added to each tube. At 660nm, absorbance was measured. In control testing, 0.05 ml water was used instead of extracts, whereas the product control was devoid of bovine serum albumin. The proportion of inhibition of denaturation of protein was estimated (Shravan,2011) as:

Percentage Inhibition = (Abs Control – Abs Sample)/Abs Control× 100

2.7.2.2. Membrane stabilization method

Sterilized Alsever solution 10ml (0.42% sodium chloride in water, 0.05% citric acid, 0.8% sodium citrate and 2% dextrose) was blended along with equivalent amount of fresh whole human blood. Isosaline was used to wash the packed cells three times after being centrifuged at 3000 rpm for 10 minutes (0.85 percent pH 7.2). Blood volume was measured and reconstituted in suspension of isosaline (10% v/v).

Heat induced hemolysis: Human Red Blood Cell membrane stabilization by membrane lysis induced by hypotonicity is the principle involved. Phosphate buffer 1ml (pH 7.4, 0.15M), HRBC suspension 0.5ml (10% v/v) along with 0.5ml test solution (6.25-100µg/ml) and hyposaline 2ml (0.36%) constituted assay mixture. standard drug used was diclofenac (6.25-100µg/ml). All reaction mixture in the centrifugation tubes were kept at 56°C for 30 min in water bath. After incubation, all tubes were placed under tap water for cooling. At 2500 rpm, centrifugation of reaction mixture was carried out for 5minutes, and at 560nm absorbance of supernatants was measured. Experiment was repeated thrice for all test samples. Percentage membrane stabilization activity was calculated by the formula as shown below (Bag *et al*.,2013).

% Hemolysis = (Absorbance of test/ Absorbance of control) x 100

%Protection = 100 – [(Absorbance of test/ Absorbance of control) x100

2.8. Statistical analysis

All the data were significantly evaluated with graph prism pad 6. the Hypothesis testing methods include ANOVA, P values of less than 0.05 were considered to indicate statistical significance. All the results are reported as the mean ± the standard error of the mean(SEM).

3. Results

3.1. Morphological studies

The morphological evaluation of *E.bicolor* Roxb. was carried out and the observations are given in figure:1. The *E.bicolor* Roxb. has a characteristic odour and bitter taste. In dry grasslands, the plant grows to a height of 25-80cm, while in upper grasslands, it grows to a height of 40-120cm. Stems quadrangular, branched basally and apically, winged (wings 0.2–0.4 cm broad), hard at the base when mature, nodes and internodes are present. Leaves are dark green, sessile-subsessile, glossy, and have broadly elliptic-ovate, spathulate or linear-lanceolate or broadly oblong lamina, 4–16 × 1.5–2.5 cm in size, 3–5 nerved at base, cuneate at base, acute apex and thick root and perennating structure.

Flowers are dichasial cyme inflorescence, terminal or axillary. At any given moment, there will be 10-20 buds to bloom and at an average there will be 40 flowers per plant. Flowering periods last for 30-45 days; pedicels 0.5–3 cm long. The flower bears a green calyx which is dorsally winged. The wings are 3-5mm broad, the calyx has 4 ovate-lanceolate lobes with 1–1.5 × 0.3–0.5 cm size and acute-acuminate apex. The corolla tube is 0.5–1 cm long yellowish white colour. The flower has 4 petals and the petals are violet colour at apex and white the rest, yellow at the throat. Stamens-4; pale green-yellow short filament, 0.3–1.5 cm long; yellowish orange anthers, linear, curved, sagittate, 1–1.8 cm long. Green ovate ovary, 0.5–1.5 × 0.3–0.8 cm; greenish white style, 1–2 cm long, deflexed; simple, rounded stigma, faintly 2-lobed; lobes are 0.15 cm long.

Fruits are capsule and brown, oblong-ovate, 1–2 × 0.8–1 cm, unilocular. Numerous, minute tetrahedral seeds, 0.2–0.35 × 0.1–0.2 mm in size with reddish brown testa. All these parameters were recorded for this plant, and these were helpful in the primary identification of *E.bicolor*.

Figure1. a) *E.bicolor* Roxb. b) Leaf of *E. bicolor* Roxb. c) Flower of *E.bicolor* Roxb.

3.2. Microscopical studies

The *E.bicolor* Roxb. leaf is dorsiventrally differentiated. Epidermal cells were present on both the upper and lower surface of the leaf. The cells in the upper epidermis are larger than those in the lower epidermis. The upper and lower epidermal cells are continuous over the midrib region. Uniseriate, multicellular covering trichomes and anomocytic stomata are present in the leaf epidermis. The laminar region of the plant has palisade cells and spongy parenchyma cells. Palisade cells were only found under the upper epidermis. The spongy parenchyma

comprises loosely arranged parenchymatous cells with vascular strands. The midrib region consists of strips of collenchyma present above the lower epidermis and below the upper epidermis. Arc-shaped collateral closed vascular bundles is more pronounced towards the midribs ventral surface. A well-developed phloem and xylem tissues are present in the dorsal and ventral surface of midrib respectively. The microscopical images were given in figure2.

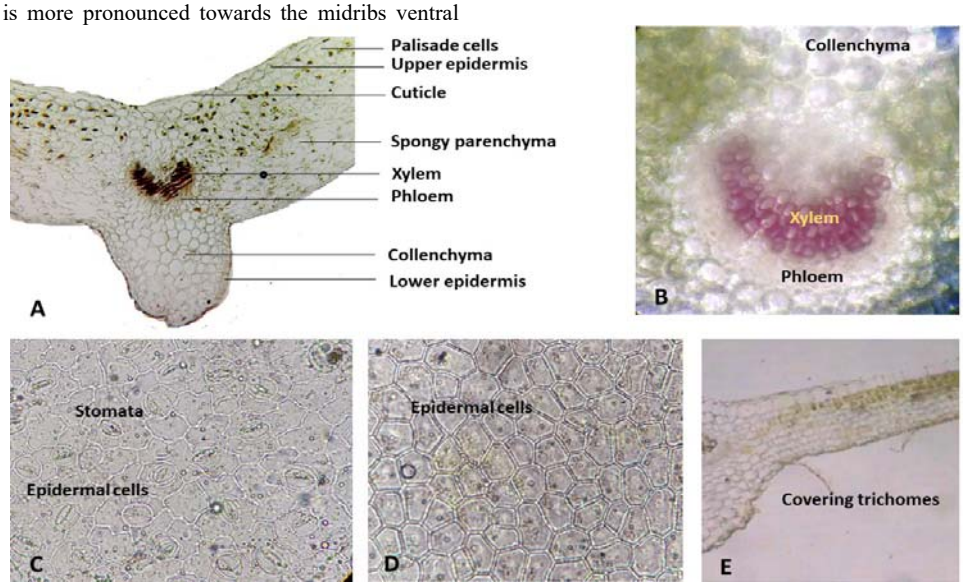

Figure 2. A) Transverse section of *E.bicolor* Roxb. leaf(60X), B) Vascular bundle(270X) C) Stomata(60X) D) Epidermal cells(60X) E) Uniseriate multicellular covering trichomes(60X)

The transverse section of the stem is quadrangular in shape without any hairs. The microscopy of the stem showed the presence of four stem wings. The outer most part is with single-layered rectangular epidermal cells with a very thick and smooth cuticle, next to the epidermis cortex is present and cortex is composed of 3-5 layers of parenchymatous cells which are loosely arranged with abundant starch grains. Thick-walled, elongated, single-layer endodermis cells without any intercellular spaces were present. The vascular bundles are present below the single-layered endodermis. The phloem is situated on the peripheral side of the xylem and is distinct. Xylem is more prominent and inhabits the chief part of the stem. The xylem possesses xylem fibres, xylem vessels and xylem parenchyma. The vascular bundles form a ring around the central pith. The large polygonal parenchymatous cells make a central pith and these cells contain calcium oxalate crystals. The microscopical images were given in figure 3.

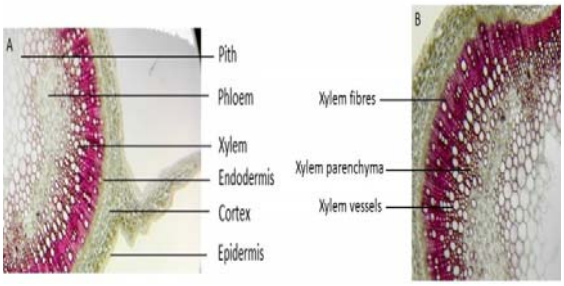

Figure 3: A) Transverse section of *E. bicolor*Roxb. stem (60X)B) Transverse section shows xylem vessels, xylem parenchyma and xylem fibres(100X)

The root of *E.bicolor* Roxb. consist of distinct, lignified cork, and below the cork few layers of phellogen and phelloderm are present. The cortex is relatively large, thick-walled parenchymatous cells containing abundant starch. The phloem encircles the xylem, and the xylem is developed with distinct vessels and lignified xylem parenchyma. Medullary rays are running between the xylem. The microscopical images were given in figure:4.

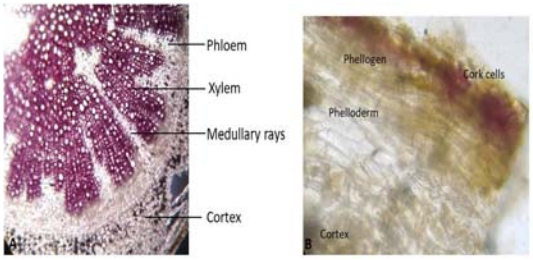

Figure 4:a) Trasverse section of periderm of *E.bicolor*Roxb. (60X)b) Transverse section of root of *E.bicolor*Roxb.(100X)

3.3. Powder microscopical studies

The microscopical evaluation of powdered *E.bicolor* Roxb. was carried out. The microscopy showed the presence of anomocytic stomata; the guard cells advance a lenticular pore in between and become bean-shaped. Polygonal, isodiametric, or elongated epidermal cells but were not arranged in a definite pattern. Uniseriate multicellular trichomes are present in the plant. The pollen grains which produce male gametes. Spiral and reticulate xylem vessels, xylem parenchyma and parenchymatous cells are also present in the powder of *E.bicolor* Roxb.. The images of different characters were given in figure:5.

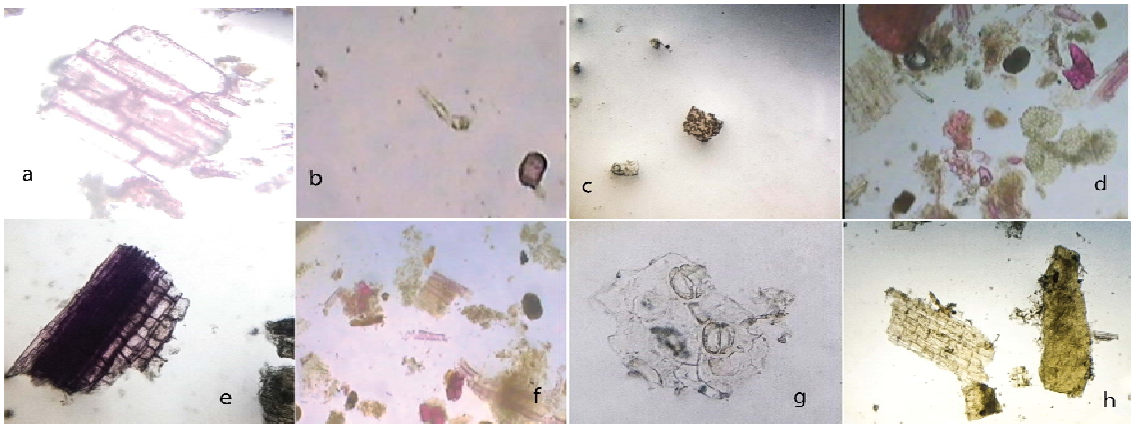

Figure 5: a)Parenchymatouscells(60X) b)Covering trichomes(60X) c)Epidermal cells(60X)d) Pollen grains(60X) e) Xylem fibres with xylem vessels(60X) f) Spiral xylem vessels(60X) g) Stomata(270X) h) Epidermal cells(60X)

3.4. Determination of leaf constant

The constants like a stomatal number, stomatal index, vein islet number and vein termination number of *E.bicolor* were determined, and the results were given in the table 1

Table 1. Leaf constant of *E.bicolor* Roxb.

Sl.no:	Leaf constant	Value per square mm
1	Stomatal number	82.2±4.970
2	Stomatal Index	24.4±3.050
3	Vein islet no	12±1.871
4	Vein termination no	7.4±1.140

Each column represents as means ± SD (n = 5)

3.5. In vitro biological assays

3.5.1. Antioxidant activity

3.5.1.1. DPPH scavenging activity

The DPPH radical scavenging activity of a plant extract is one of the most broadly used methods for determining its antioxidant activity. DPPH is a protonated radical with a characteristic absorption peak at 517 nm that lowers as the proton radical is scavenged by natural plant extracts. The ability of various extracts of *E. bicolor* to scavenge DPPH free radicals was assessed by measuring the decrease in DPPH absorbance at 517 nm. The activity of DPPH radical scavenging was measured in percentage inhibition. The chloroform extract showed a better antioxidant activity compared to other extracts. Ascorbic acid was used as standard. Ascorbic acid(standard) IC_{50} value was found to be 14.09±0.636µg/ml followed by chloroform extract 67.46±0.916µg/ml, acetone extract 88.52±2.431µg/ml, methanol extract 96.60±3.516µg/ml and aqueous extract 176.09±8.934µg/ml. The results were given in figure 6.

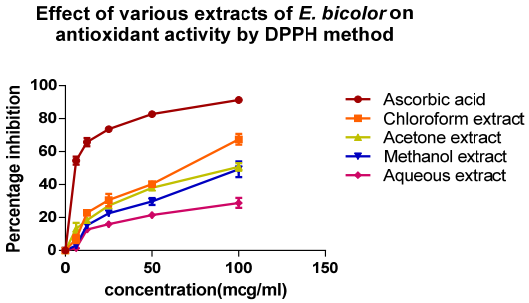

Figure 6. Effect of various extracts of *E.bicolor*Roxb. on antioxidant activity by DPPH method.

3.5.1.2. Nitric oxide scavenging assay

Nitric oxide scavenging activity was carried out with various extracts (chloroform, acetone, methanol and aqueous) of *E.bicolor* and ascorbic acid as standard compound. The reductive potential of all extracts and standard preparations exhibited dose-dependent activity. Ascorbic acid(standard)IC_{50} value was found to be 21.94±0.478µg/ml followed by chloroform extract 63.92±0.916µg/ml, acetone extract 92.52±1.987µg/ml, methanol extract 106.85±2.035µg/ml and aqueous extract 121.50±2.512µg/ml. The results are given in figure 7.

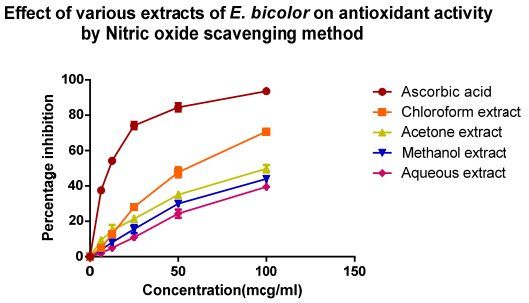

Figure 7. Effect of various extracts of *E.bicolor*Roxb.on antioxidant activity by nitric oxide scavenging method.

3.5.2. Anti-inflammatory activity of whole plant extracts

3.5.2.1. Inhibition of protein denaturation method

In this regard, the chloroform, acetone, methanolic and aqueous extracts of the whole plant of *E.bicolor* displayed significant activity. The methanolic extracts at a concentration of 100µg/ml showed maximum activity among all extracts. The IC_{50} value of standard diclofenac sodium was found to be 26.56±1.100µg/ml followed by methanol extract 56.06±0.735µg/ml, chloroform extract 74.99±1.972µg/ml, acetone extract 88.63±2.459µg/ml and aqueous extract 119.89±4.891µg/ml. The results are given in figure 8.

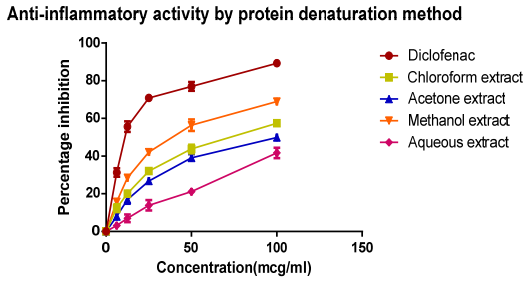

Figure 8. Effect of various extracts of *E.bicolor*Roxb. on anti-inflammatory activity by protein denaturation method.

3.5.2.2. Membrane stabilization test

Human red blood cell membrane lysis inhibition induced by hypotonicity,i.e. stabilization of HRBC membrane, was taken as a degree of the anti-inflammatory activity. The percentage of membrane stabilization for various (chloroform, acetone, methanol and aqueous) extracts of *E.bicolor* was done. Diclofenac sodium was used as standard, and it showed a percentage protection of 83.95±1.487 at 100µg/ml. The methanolic extract was effective in inhibiting the heat-induced hemolysis of HRBC at different concentrations compared to other extracts. The percentage protection of methanolic extract was found to be 67.85±1.113 at 100µg/ml, chloroform 49.67±1.068 at 100µg/ml, 46.83±2.801 at 100µg/ml and acetone 45.39±1.391 at 100µg/ml. The results are given in figure 9.

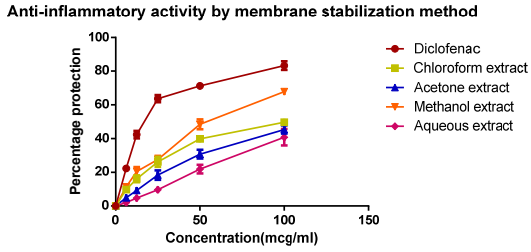

Figure 9. Effect of various extracts of *E.bicolor*Roxb.on anti-inflammatory activity by membrane stabilization method.

4. Discussion

Despite the extensive medicinal uses of *E. bicolor* Roxb., there is little information on the morphological and microscopical parameters that would justify the quality control profile of this plant. Herbal drug standardization is critical for confirming the safety and efficacy of herbal drugs. Organoleptic and microscopical evaluation are the parameters for standardizing crude drugs. Morphological studies are the quickest means to evaluate the identity and purity of any crude drug. In addition to morphology, microscopic features that can be easily distinguished are equally important in the confirmation of the identity and purity of the plant. The leaf of *E.bicolor* Roxb.is dorsiventral type; it consists of covering trichomes and anomocytic stomata. The stem is quadrangular in shape and shows the presence of four wings. Besides, the stem consists of the epidermis, cortex, endodermis, phloem, xylem, and pith. Xylem is more prominent and occupies a significant portion of the stem. The *E.bicolor* Roxb. root consists of evident, lignified cork, and a few layers of phellogen, and phelloderm is present beneath the cork. The cortex is relatively large, and the phloem encircles the xylem. The xylem is developed with distinct vessels and lignified xylem parenchyma. Medullary rays are running between the xylem.

Free radicals are recognized to play a significant role in a wide range of clinical manifestations. By combating various free radicals, antioxidants protect us from a multiple ailment. They either work by conserving antioxidant defense systems or scavenging reactive oxygen species (Umamaheswari and Chatterjee, 2008). The ability of natural products to donate electrons can be assessed using the 2, 2′-diphenyl-1-picrylhydrazyl radical (DPPH) purple-colored solution bleaching method (Nunes*et al* 2012. Al-Ghamdi*et al.*, 2020). Chloroform extracts had a substantially higher inhibitory percentage than the other fractions evaluated in this study. This study implies that phytochemical elements in the plant extract can donate hydrogen to a free radical to scavenge potential harm, as given in figure 6.

Endothelial cells, macrophages, and neurons all produce nitric oxide, which is involved in the control of a variety of physiological processes, including inflammation. Excessive nitric oxide generation and release have been linked to several disorders such as cardiovascular and inflammatory diseases, cancer and cataract. Specific nitric oxide synthase (NOSs) produces nitric oxide in biological tissues by metabolizing arginine to citrulline and forming nitric oxide via a five-electron oxidative process. These substances alter many cellular component's structures and functional behavior (Knowles, 1986). The drop in absorbance at 546 nm caused by a reduction in nitric acid generation was used to measure the extract's nitric oxide scavenging potency.

The anti-inflammatory action of medicinal plants was investigated using protein denaturation and stabilization of human red blood cell membranes. Protein denaturation is commonly related to inflammation (Naz*et al*.,2017). The current research found that methanolic extract considerably reduced protein/albumin denaturation.

The erythrocyte membrane is identical to the lysosomal membrane; hence, the extract may also be able to stabilize lysosomal membranes. This stabilization is critical in restraining the inflammatory response because it prevents the extracellular release of lysosomal contents of activated neutrophils such as proteases and bactericidal enzymes, which induce additional tissue inflammation and damage (Azeem*et al*.,2010). Hypotonicity-induced hemolysis can be caused by cell shrinkage caused by osmotic loss of intracellular electrolytes and fluid components. The extract

may inhibit or promote mechanisms stimulating or enhancing intracellular component efflux. (Kumar *et al*.,2012).

Furthermore, the findings by Rajisha *et.al* 2020a., alkaloids, glycosides, phenolic compounds, flavonoids, sterols, carbohydrates, terpenoids, saponins and gums, and mucilage are present in *E.bicolor* Roxb. The flavonoids and phenolic compounds in the extract may be responsible for this antioxidant and anti-inflammatory activity.

5. Conclusion

According to Ayurvedic Pharmacopoeia of India, morphological and microscopical studies are vital parameters for standardizing crude drugs. Morphological studies are the quickest means to ensure the identity and purity of any crude drug. Distinguishable microscopical characters are also very helpful for confirmation of the identity and purity of the plant. The authenticity of any crude drug needs to be standardized using a suitable method to prevent adulteration. *E.bicolor* Roxb. is a dicot plant. This study can be an essential tool for the botanical identification of the plant *E.bicolo r*Roxb. since no comprehensive standardized work has been stated in the literature for this plant so far. Therefore, this study gives exclusive authenticity parameters, which will help conscript a monograph of this plant. The present study also supports the antioxidant and anti-inflammatory activity of *E.bicolor* Roxb.

Acknowledgment

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