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INVESTIGATION ON BIOCHEMICAL AND ANTIMICROBIAL POTENTIAL OF EUPATORIUM ADENOPHORUM, A MEDICINAL PLANT SPECIES FROM WOKHA NAGALAND, INDIA

Benchilo Ezung[1]*, Ranjan Dutta Kalita[2] and Agastian Paul[1]

[1]Research Department of Plant Biology and Biotechnology, Loyola College, Chennai-34, Tamil Nadu, India.

[2]Department of Biotechnology, Royal School of Biosciences, Assam Royal Global University, Betkuchi, Guwahati- 781035 Assam, India.

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***Corresponding Author**
Benchilo Ezung
Research Department of Plant Biology and Biotechnology, Loyola College, Chennai-34, Tamil Nadu, India.

ABSTRACT

Throughout the course of history, plants and their derived extracts have been utilized for therapeutic purposes, spanning across numerous millennia. Even in contemporary times, medications derived from plants remain integral components of global healthcare systems. *Eupatorium adenophorum* is a significant botanical species with medicinal properties, which is recognized as being susceptible to environmental pressures in several regions across the globe. The current investigation focused on examining the antibacterial activity, antioxidant assay, total phenols, alkaloids content, and haemolysis activity of the plant species. The antibacterial activity of the ethanol extract of *Eupatorium adenophorum* was shown against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with a minimum inhibitory concentration (MIC) of 2.5 mg/ml. The extract exhibited a significant level of antioxidant activity, measuring approximately 68.39%. The analysis revealed the presence of a significant concentration of alkaloids in the extract. The phenolic content of the extract was found to be significantly low, and no haemolytic activity was observed, suggesting that the extracts possess non-cytotoxic properties. Therefore, based on the findings of this study, it is recommended that more research be conducted to explore the antibacterial and antioxidant capabilities of *Eupatorium adenophorum*.

KEYWORDS: *Eupatorium adenophorum*, medicinal plants, antioxidant, antimicrobial, MIC.

INTRODUCTION

During the pre-modern era, the traditional system of medicine had amassed a substantial body of knowledge pertaining to several species of medicinal plants. The accumulation of knowledge across multiple generations within diverse communities has been extensively explored.[1] The utilization of alternative therapies and herbal medicines has witnessed a significant surge in popularity, leading to a substantial rise in the demand for medicinal plants and their derivatives. This trend persists despite the advancements made in modern medicine for both preventive and curative treatments, observed in both developed and developing nations. The state of Nagaland, located in Northeast India, boasts a significant level of biodiversity. As a result, the traditional therapeutic practices employed by folk practitioners in the region heavily rely on the utilization of locally sourced plant species for health management purposes. The utilization of several types of wild edible plants, fruits, and medicinal plants by the tribal group residing in these hills serves as a clear indication of the abundant plant diversity present in the region.[2]

Eupatorium adenophorum, a member of the Asteraceae family, plays a significant role as a colonizer in early succession communities that emerge following Jhums (shifting farming) at the high elevations of the North-eastern hill area of India. Numerous accounts exist about the utilization of the entire plant, encompassing both leaves and shoots, as medicinal remedies within various regions across the globe.[3] *E. adenophorum* is a substantial genus including of herbs, shrubs, or under shrubs, primarily found in tropical regions of the Americas. The Asteraceae family exhibits a broad distribution throughout various regions, including China, New Zealand, India, Nepal, Pakistan, Thailand, Malaysia, Philippines, Singapore, eastern Australia, northern America, and South Africa.[4] The plant is a perennial herbaceous species that typically grows in an upright manner, reaching a height ranging from 1 to 2 m. *E. adenophorum* possesses notable characteristics that enable it to thrive in challenging environments, such as arid and exposed slopes, roadside areas, abandoned fields, nutrient-deficient wastelands, and degraded locations like Jhums fallows. It can adapt to land that has remained untended or unsown for extended periods following vegetation burning, as well as degraded rocky surfaces. However, it does not typically flourish in shaded forest environments. The recovery process is rapid, especially in altitudes where winter frost is

present. The species has been documented to exhibit growth at a diverse range of altitudes, specifically between 850 and 2,050 m above sea level. It demonstrates a preference for acidic soils, with a pH range of 5.4 to 6.1.[5] Despite being utilized in traditional practices, it is important to acknowledge that this particular phenomenon also carries a drawback of rapid dissemination, resulting in considerable harm to both the ecological environment and economic progress.[6] A majority of plant species undergo the preparation of crude drugs in the form of aqueous extracts. It is noteworthy to observe that the utilization of leaf was predominant in the majority of instances.[7] The species *E. adenophorum* has been utilized in traditional medicinal practices, such as ayurveda and other folk remedies, for many therapeutic purposes. These include its application in the treatment of cuts and wounds, as well as its usage as a haemostatic and antifungal agent.[8] The plant is utilized in traditional medicine for its antiseptic, blood coagulant, antipyretic, antibacterial, and analgesic properties.[9] The objective of this study is to assess the biochemical makeup and antibacterial capabilities which can assist the researcher in their subsequent investigations pertaining to this plant species.

MATERIALS AND METHODS

Plant Material and Preparation of extract

The plant material of *E. adenophorum* had been collected from Wokha, Nagaland, India, specifically during the month of October. The plant underwent authentication by Dr. Bhabananda Baruah, a taxonomist and botanist. A voucher specimen was submitted to the herbarium at the University of Science and Technology, Meghalaya.

Figure 1: *Eupatorium adenophorum*.

The leaf of the plant were gathered and afterwards subjected to a washing process, followed by a drying procedure conducted in shaded conditions. The leaf were thoroughly dried and

afterwards pulverized into a fine powder using a laboratory grinder. The dried leaf powder was placed in a hermetically sealed plastic bag and thereafter stored at a temperature of -4°C until it was to be utilized for subsequent purposes. A quantity of 500 g of dry powder was placed into a beaker and subsequently combined with 1.5 L of ethanol. The mixture was then agitated continuously for a duration of 72 hours, equivalent to three days. The content underwent filtration using Whatman filter paper no.1. The resultant extracts were subjected to a drying process and thereafter kept in small aliquots at a temperature of approximately 4°C.

Bacterial strains

The microorganisms employed in this study are *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which serve as representatives of Gram-positive and Gram-negative bacteria, respectively. Bacterial stock cultures were generated by suspending the bacteria in glycerol and thereafter storing them at a temperature of -80°C.

Antimicrobial assay

The antimicrobial assay was conducted using the methodology outlined in the study by Rangaswamy *et al.*[10] The Minimum Inhibitory Concentration (MIC) experiment was conducted using the micro dilution technique on a 96-well plate. The initial well was filled with the extract at a stock concentration of 10mg/ml. Serial dilutions were performed starting from the second well and continued up to the tenth well. A volume of 100µl of the fresh broth was added to each well from the second to the twelfth. A volume of 20µl from the 0.4 optical density (OD) cultures was introduced into each well, followed by an overnight incubation at a temperature of 37°C. A solution of MTT (5mg/ml) was utilized as a dye and introduced into the wells in a volume of 20µl. The samples were thereafter incubated for approximately 1 hour. The alteration in the color pattern demonstrated the antibacterial efficacy of the crude extracts. In the event that a yellow color change occurs, it can be inferred that antimicrobial activity has been established. Conversely, if the color remains blue, it suggests the presence of bacterial survival.

Antioxidant assay

The antioxidant assay was conducted using the DDPH radical scavenging assay, as described by Sharififar *et al.*[11] The antioxidant assay employs the DPPH radical as a reagent. The reduction of DPPH occurs upon its reaction with an antioxidant molecule. The alteration in hue (transitioning from a dark violet shade to a pale yellow shade) is afterwards quantified. A volume of 50µl of the sample, dissolved in methanol, was introduced into 5ml of DPPH

solutions containing 0.004% methanol. Four distinct concentrations (1mg, 0.5mg, 0.25mg, 0.125mg) of the ethanol solvent extract derived from the leaves of *E. adenophorum* species were utilized. The spectrophotometer used for this experiment was a Thermo Fischer model. After a 30-minute incubation period in darkness at room temperature, the absorbance was measured at a wavelength of 517nm. The calculation of the percentage inhibition (I) of the DPPH free radical was performed using the following formula: I% = (A blank – A sample/ A blank) ˣ100

The variable 'A blank' represents the absorbance value of the control reaction, which includes all the reagents except for the extract. The term "sample" refers to the absorbance value of the extract, which represents the test substance. The standard used in this study was ascorbic acid. The determination of the concentration of the extract that resulted in 50% inhibition was derived by analyzing the graph that depicted the relationship between inhibition concentration and extract concentration.

Total phenols assay

The determination of the Total phenolic content was conducted using the methodology outlined by Chumark *et al*.[12] with a little variation that involved the use of Gallic acid as the standard. A volume of 10 ml of 40% ethanol was added to 5 mg of the extract, followed by sonication for duration of 30 min. The sample was subjected to vortexing for duration of approximately two minutes, followed by a period of one hour of standing at ambient room temperature. A volume of 100µl of the extract was transferred into a test tube, followed by the addition of 6ml of distilled water. The solution was agitated by swirling, followed by the addition of 500µl of Folin Ciocalteu's phenol reagent and subsequent mixing. Following a time interval of five minutes, a volume of 1.5 ml of a sodium carbonate solution with a concentration of 20% was introduced. The components were combined and the resulting solution was diluted to a final amount of 10ml using distilled water. The resulting extract sample was subsequently subjected to vortexing one more. The solution was subjected to incubation at ambient temperature for duration of approximately two hours. The measurement of absorbance was conducted at a wavelength of 760nm. Different quantities of Gallic acid (50µl, 100µl, 200µl, 400µl) were employed as standard solutions.

Alkaloids assay

Mayer's reagent

This test serves as an additional method for confirming the presence of alkaloids. The reagent was made by dissolving 1.358 g of mercury chloride (HgCl2) in 60 ml of distilled water, followed by the addition of this solution to a separate solution containing 5 g of potassium iodide (KI) dissolved in 10 ml of pure water. Subsequently, an appropriate quantity of distilled water was introduced to modify the solution's volume to 100ml.

Wagner's reagent

It is the second test for alkaloids. Here, 2g of iodine and 6g of potassium iodide (KI) was dissolved in 100ml of distilled water.

Haemolysis assay

The Haemolysis test was conducted following the methodology outlined by Nair *et al.*[13] Blood samples from a goat were obtained and stored in a container containing a solution of trisodium citrate with a concentration of 4%. The sample underwent centrifugation at a speed of 750 revolutions per minute (rpm). The liquid portion was removed and the solid material containing the red blood cells was rinsed with phosphate-buffered saline (pH 7.4) two times at a centrifugal speed of 750 revolutions per minute for duration of 10 minutes. A total volume of 48.5ml of phosphate-buffered saline (PBS) was combined with 1.5ml of the erythrocyte suspension, resulting in a final erythrocyte concentration of 3%. A volume of 1.9 ml of the suspension was introduced into a centrifuge tube with a capacity of 2 ml. A volume of 100µl of the extracts was introduced into the tube, with a concentration of 1mg/ml. The mixture consisting of the extract and suspension was subjected to incubation at a temperature of 37°C within an incubator. Following the incubation period, the tubes underwent centrifugation at a speed of 750 revolutions per minute (rpm) for duration of 10 minutes. A volume of 200µl of the supernatant was transferred into a new tube, followed by the addition of 2.8ml of phosphate-buffered saline (PBS). The resulting mixture was subjected to absorbance measurement at a wavelength of 415nm using a spectrophotometer manufactured by Thermo Fischer. The negative control in this experiment was PBS, while the positive control was Triton X 100.

RESULTS AND DISCUSSION

E. adenophorum has been utilized as a traditional medicinal plant throughout several regions since ancient times. In numerous underdeveloped regions, indigenous healers employ it as a

means of pain relief, a substance that inhibits the growth of cells, a fever reducer, and an agent that combats microorganisms, a substance that prevents infection, and a compound that promotes blood clotting. Traditionally, the utilization of *E. adenophorum* juice or herbal tea has been documented as a remedy for several conditions affecting the stomach, liver, gallbladder, diabetes, cancer, and general discomfort.[14] The antibacterial activity of the crude ethanol extracts derived from *E. adenophorum* was examined against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The activity of the ethanol crude extract was evaluated using the minimum inhibitory concentration (MIC) assay (Figure 2).

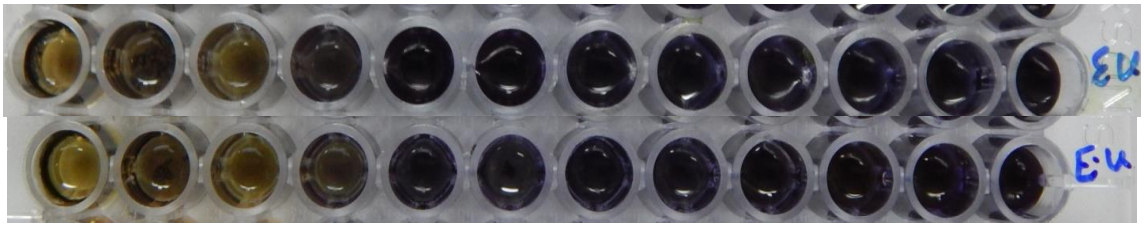

Figure 2: MIC assay of the ethanol extract against both *Pseudomonas Aeruginosa* and *Staphylococcus aureus.*

The results of the Minimum Inhibitory dosage (MIC) assay indicated that the growth of the bacteria was suppressed by the extract at a dosage of 2.5 mg/ml in both microbial samples. Therefore, the assay provided confirmation of the antibacterial activity exhibited by the ethanol extract. Moreover, it has been shown that the ethanol extract possesses potential for additional exploration in order to ascertain the specific antimicrobial compounds or components accountable for its observed activity. Some investigation showed that the leaf extract derived from *E. adenophorum* exhibits potent inhibition against tobacco mosaic virus infection, accompanied by a modest therapeutic effect. The antiviral process encompasses both the direct suppression of virus particles and the stimulation of a response inside the host plant.[15] In a separate investigation, it was noted that photochemical derived from the root of *E. adenophorum* exhibited bacteriostatic properties against three Gram-positive bacteria of the minimum inhibitory concentration (MIC) values ranged from 12.5 to 50µg/ml.[16] The methanol extract of *E. adenophorum* exhibited *in vitro* antibacterial activity against MRSA, with a MIC of 12.5 mg/ml, and against *S. aureus* ATCC 25923, having a MIC of 25 mg/ml.[17]

Oxidative stress induced by environmental free radicals, results in cellular damage by modifying physiological components such as proteins and DNA. The compound that acts as a scavenger for free radicals is commonly referred to as an antioxidant. The conducted study on

antioxidants, utilizing the DPPH radical scavenging assay, revealed that the extract exhibits a substantial level of antioxidant activity. The radical scavenging activity of the ethanol extract derived from *E. adenophorum* species is approximately 68.39%, whereas the positive control Ascorbic acid had a higher activity of 92.05%. Hence, it is plausible that the aforementioned extract could serve as a viable anti-inflammatory agent, contingent upon the identification of the specific components within the extract that exhibit anti-oxidant capabilities. The extract's scavenging activity can be evaluated from the graph depicted in Figure 3. The total antioxidant capacity (TAC) of the leaves of *E. adenophorum* was seen to vary from 16.98% to 94.87% of the standard ascorbic acid when the plant extract was diluted in methanol, throughout a concentration range of 0.05 to 1 mg/ml.[18] Therefore, the potential application of *E. adenophorum* leaf as a natural antioxidant in the future is deemed significant.

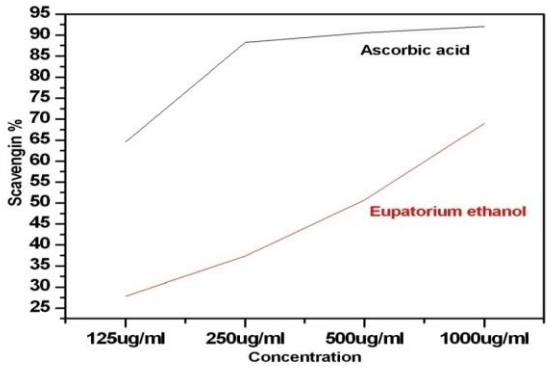

Figure 3: DPPH radical scavenging assay of the ethanol extract.

Medicinal plants possess a diverse array of bioactive chemicals, rendering them a significant reservoir for the discovery and development of novel therapeutic medicines with unique features. The pharmacological characteristics of medicinal plants are primarily determined by the presence of many kinds of phytochemical substances, such as phenolic compounds, flavonoids, glycosides, tannins, lignans, glycosides, terpenoids, proteins, furocoumarins, alkaloids, resins, steroids, and peptides.[19] *E. adenophorum* was found to decrease the levels of antioxidants, including superoxide dismutase, catalase, glutathione peroxidase, and glutathione. Conversely, there was a significant increase in the levels of reactive oxygen species and malondialdehyde. These changes resulted in the induction of oxidative stress and pyroptosis, ultimately leading to impaired spleen function in mice.[20] The quantification of the overall phenolic content was conducted using Gallic Acid Equivalent (GAE) as the standard unit of measurement. However, it was revealed that the ethanol extract derived from *E. adenophorum* did not exhibit any phenolic content. The ethanol extract exhibited a

significant quantity of alkaloids, as evidenced by the positive results obtained from both the Wagner's test and Mayer's test.

The haemolysis experiment was conducted in order to determine whether the extract caused any detrimental effects on the red blood cells. Nevertheless, the analysis revealed that the extracts had no haemolytic activity (Figure 4), indicating that the extract does not possess cytotoxic properties. The various concentrations of extracts did not exhibit any haemolytic effects in comparison to the positive control, triton X100. Recent research has shown significant interest in the phytochemical and pharmacological investigations conducted on extracts derived from various components of *E. adenophorum*, as well as the isolation and study of chemicals obtained from this plant. This review encompasses a diverse range of bioactive chemical elements and their corresponding pharmacological actions, which encompass anti-inflammatory, analgesic, antipyretic, antioxidant, antibacterial, antifungal, anticancer, and other properties. There is a growing body of evidence that substantiates the potential use and exploration of *E. adenophorum* for the purpose of developing novel pharmaceuticals. Nevertheless, it is advisable to refrain from the oral use of *E. adenophorum* and its derivatives without appropriate dosage due to the well-documented hazardous properties associated with this plant. Additionally, it is necessary to conduct further research on the long-term toxicity and other toxicological factors associated with *E. adenophorum*. Further investigation and verification are required to examine the correlation between the molecular structures of the bioactive chemicals derived from *E. adenophorum* and their diverse pharmacological actions.

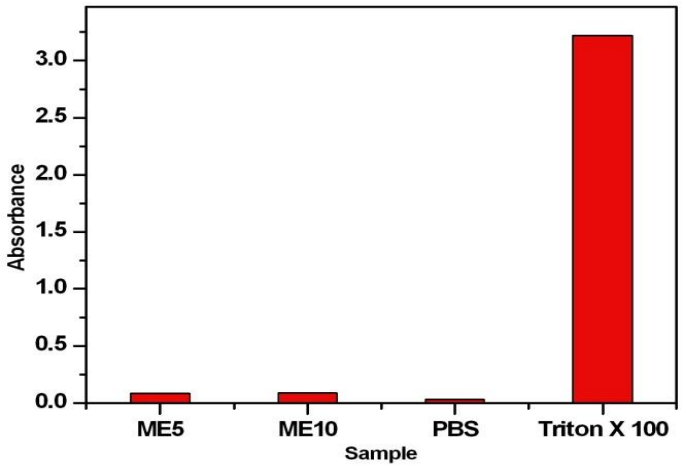

Figure 4: Haemolysis assay of the ethanol extract.

CONCLUSION

The current investigation focused on the ethanol extract of *E. adenophorum*, aiming to determine its antibacterial, antioxidant, and haemolytic activities, as well as analyze specific biochemical components of the plant. The antibacterial activity of the ethanol extract derived from *E. adenophorum* was observed against the microorganisms employed in this investigation. A minimum inhibitory concentration (MIC) of 2.5mg/ml was observed for both *P. aeruginosa* and *S. aureus*. The ethanol solvent extract exhibited an antioxidant activity above 68.39%. The extract did not exhibit any phenolic content, which was consistent with its antioxidant activity in comparison to the positive control, ascorbic acid. The analysis did not reveal any evidence of haemolysis in the red blood cells, suggesting that the extracts did not exhibit cytotoxic effects. The presence of alkaloid content in the extract was confirmed. Therefore, based on the findings of our study, it can be inferred that there is potential for future exploration of the antibacterial and antioxidant capabilities of *E. adenophorum*. Additionally, it is necessary to conduct research on the mechanisms of action and ingredients responsible for the therapeutic effects exhibited by this plant. This plant possesses significant potential as a medicinal resource, since it can be further investigated for the identification of novel medications and drug targets that combat a wide range of disease-causing substances. Consequently, it holds substantial significance in the field of medicine.

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