Phytochemical and Pharmacological Evaluation of *Andrographis Paniculata* and *AdhatodaVasaka*

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KEYWORDS
AndrographisPaniculata, *AdhatodaVasaka*, antibacterial, anti-inflammatory, antioxidant, phytochemical screening, toxicity evaluation, pharmacology.

ABSTRACT:
This prospective study researching the phytochemical and phytochemical properties of *AndrographisPaniculata* and *AdhatodaVasaka*, which are well known medicinal plants. The leaves of both plants were subsequently prepared into methanolic extracts and screened for phytochemicals such as alkaloids, flavonoids, terpenoids, saponins, tannins and glycosides. The antibacterial activity of both extracts was strongly shown, *AndrographisPaniculata* inhibits Staphylococcus aureus while *AdhatodaVasaka* is more effective in *Escherichia coli*. The ability of *Andrographis Paniculata* to possess anti-inflammatory activity was evaluated by measuring its effect on the carrageenan induced paw oedema model in Rhythas. Free radical scavenging activity was assessed by the DPPH assay using *Andrographis Paniculata* having stronger antioxidant potential than Datura stramonium. Both extracts were discovered to be nontoxic at high doses. These results offer promise that both plants might have pharmacological value in infections, inflammation and oxidative stress treatments but further research is required to clarify how.

1. Introduction

Two well-known medicinal plants *Andrographis Paniculata* (commonly known as the King of Bitters) and *AdhatodaVasaka* (Vasaka) are widely used in traditional medicine for their multiple therapeutic applications. They have a special effectiveness in treating respiratory, inflammatory, and microbial sickness. Andrographolide, the bioactive compound of, *Andrographis Paniculata* is considered to have robust anti-inflammatory, antimicrobial, antioxidant, antidiabetic, and immunomodulatory properties (Choi et al., 2017; Kumar, et al., 2020). This plant has been used traditionally in Ayurveda to manage fever, upper respiratory infections, and inflammation, and studies of its hepatoprotective and anticancer activities (Basak et al., 1999; Adedapo et al., 2015). However, *AdhatodaVasaka*, which is widely used for its alkaloid vasicine and which has been a mainstay of treatment for respiratory conditions such as asthma, bronchitis, and chronic cough. The pharmacological profile of herb includes an effect as bronchodilator, expectorant, anti-inflammatory, and an antimicrobial (Singh et al., 2020). This multifaceted therapeutic potential of these plants suggests that their phytochemical and pharmacological properties have to be scientifically validated before their further use in modern medicinal systems (Mitra&Kannan, 2007; Valdiani et al., 2012).

Rigorous experimental evaluation of the *AndrographisPaniculata* and *AdhatodaVasaka* through experimental means is made in this study with regards to their antibacterial, antiinflammatory and antioxidant properties. Findings aim to plug this gap between traditional knowledge and scientific evidence, giving a solid foundation for future drug development (Sen & Sen, 2023; Kumar et al., 2013). This research evaluated the efficacy of the plants in combating bacterial infections, oxidative stress and inflammation and thus is an ongoing effort to identify plant based therapeutic agents with minimal toxicity profiles (World Health Organization, 1990; Ahmad et al., 1992).

2. Literature Review

2.1 Phytochemistry of *Andrographis Paniculata*

Andrographolide, a bioactive compound present in *Andrographis Paniculata* (excellent source of resistant starch) has been well investigated in potential therapies. The potent anti-inflammatory and antimicrobial properties of the plant are due to Andrographolide a diterpenoid lactone (Choi et al., 2017; Lalrinzuali et al., 2016). Results from these studies indicate that the plant has shown very high antioxidant activity associated with the capability to eliminate free radicals as well as to combat oxidative stress (Adedapo et al., 2015; Kumar et al., 2020). With recent studies, it has been traditionally used to treat the respiratory disorders, i.e. common colds and bronchitis, with its immunomodulatory and hepatoprotective effects (Joshi et al., 2019; Tiwari, 2017). Additionally, proprotein convertases are known to have been inhibited by *Andrographis Paniculata*, which has been implicated in its antiinflammatory effects at a molecular level (Basak et al., 1999).

2.2 Phytochemistry of *AdhatodaVasaka*

Highly vasicine containing *AdhatodaVasaka* has a wide spectrum of pharmacological activities. *AdhatodaVasaka* is an important agent in respiratory therapies on account of bronchodilatory and expectorant properties, and contains vasicine quinazoline alkaloid (Sugiura et al., 1989; Subhose et al., 2005). It has been traditionally used to treat asthma and cough; in addition, the plant has also shown antimicrobial and anti-inflammatory activities due to its alkaloid rich composition (Singh et al., 2020; Tiwari & Singh, 2004). Recent investigations have also examined its antiviral properties as a natural remedy of infectious diseases (Sharma et al. 2021). *AdhatodaVasaka* is a promising candidate for therapeutic applications through its synergistic effects of its phytochemicals towards respiratory and inflammatory conditions (Das & Panda 2017; Hijazi et al. 2017).

2.3 Pharmacological Evaluation

The pharmacological activities of *AndrographisPaniculata* and *AdhatodaVasaka* have been shown extensively both in preclinical and clinical studies. Familiarly known as the *Andrographis Paniculata*, it has been found effective in treating respiratory infections, especially for its anti-inflammatory properties (Yimer et al., 2020; Kumar et al., 2020). In addition to its antioxidant activity, it further potentiates its therapeutic potential in the mitigation of oxidative damage in chronic diseases (Ahmad et al., 1992; Kumar et al., 2013). On the other hand, *AdhatodaVasaka* possesses excellent bronchodilatory and antimicrobial activities and is therefore the primary participant in respiratory disorders (management, Sugiura et al., 1989; Statile et al., 1988). Studies have shown that both plants have no toxic effects even when tested on high doses (Sen & Sen, 2023; Patel et al. 2016).

2.4 Integration with Modern Medicine

AndrographisPaniculata and Adhodavasaka have been integrated into modern therapeutic regiments as its diverse pharmacological activities and safety profile supportes this. The anticancer potential of the plant and their potential to fight bacterial infections, reduce inflammation and alleviate oxidative stress is such anti synthetic drugs and it is necessary its use. On the other hand, another mechanism based basis of their efficacy arises from the fact that they are able to interact with the molecular pathways implicated in the regulation of inflammation and oxidative stress as applicable for use in the tailored medicine (Kumar et al., 2020; Lalrinzuali et al., 2016).

This extensive review featuring the phytochemical richness and pharmacological versatility of *AndrographisPaniculata* and *AdhatodaVasaka*, to benefit from their traditional uses and pursue their potential applications in modern medicine. This study emphasizes the use of these plants as a source of natural, safe and effective therapeutic agents (Mitra & Kannan 2007; Divya et al. 2021) by synthesizing the traditional knowledge with the scientific evidence. While more research, in the form of controlled clinical trials, is required to fully undescribe their mechanism of action and optimize their therapeutic potential, this initial knowledge should be of great interest and encouragement to both researchers and the few institutions with the necessary resources and technologies.

3. Materials and Methods

3.1 Plant Collection

Fresh leaves of *AndrographisPaniculata* and *AdhatodaVasaka* were collected from the local regions of India, specifically from well-known herbal plant cultivation areas. The plants were authenticated by a botanist at the local herbarium, and voucher specimens were deposited for reference. The collected leaves were carefully cleaned to remove any debris, air-dried in a well-ventilated area, and subsequently powdered for further analysis.

3.2 Preparation of Extracts

A total of 500 g of dried and powdered leaves from each plant was subjected to solvent extraction using methanol through the maceration method. The powder was soaked in methanol for 72 hours with intermittent shaking, followed by filtration through Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure using a rotary evaporator, yielding crude methanolic extracts. These extracts were stored in airtight containers at 4°C for further investigation.

3.3 Phytochemical Screening

Preliminary phytochemical screening was performed according to standard methods (Harborne, 1998) to identify the major classes of bioactive compounds in the plant extracts:

- **Alkaloids**: Detected using Dragendorff's reagent.
- **Flavonoids**: Presence was confirmed by the alkaline reagent test.
- **Saponins**: Tested via the foam test.
- **Terpenoids**: Identified using the Liebermann-Burchard test.
- **Tannins**: Assessed using the ferric chloride test.
- **Glycosides**: Tested using the Keller-Kiliani test. All tests were carried out in triplicates, and the presence or absence of each compound was noted.

3.4 Pharmacological Evaluation

Antibacterial Activity: The antibacterial properties of the extracts were assessed using the agar well diffusion method. Bacterial strains, *Staphylococcus aureus* and *Escherichia coli*, were selected based on their relevance to common infections. The inhibition zones were measured in millimeters (mm), and the results were compared to those of standard antibiotics like ciprofloxacin.

Anti-inflammatory Activity: Anti-inflammatory effects were evaluated in vivo using the carrageenan-induced paw edema model in male Wistar rats (150-200 g). The rats were divided into four groups, including control and treated groups, with *AndrographisPaniculata* and *AdhatodaVasaka* administered at doses of 200 mg/kg orally 30 minutes before carrageenan injection. Paw volume was measured using a plethysmometer at baseline and at 1, 3, and 5 hours post-carrageenan injection. The percentage of inhibition of edema was calculated as follows:

$$Percentage\ Inhibition = \left(\frac{Edema\ in\ control - Edema\ in\ treated}{Edema\ in\ control} \right) \times 100$$

Antioxidant Activity: The antioxidant potential of the plant extracts was evaluated by the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. Different concentrations (25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL) of the plant extracts were incubated with DPPH solution. The absorbance was recorded at 517 nm, and the percentage scavenging activity was calculated using the formula:

$$DPPH\ Scavening\ Activity = \left(\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \right) \times 100$$

Toxicity Evaluation: Acute toxicity studies were conducted to evaluate the safety profile of the plant extracts. Rats were divided into groups and treated with varying doses of the extracts (500 mg/kg, 1000 mg/kg, and 2000 mg/kg). Observations for any behavioral changes, mortality, weight fluctuations, and organ morphology were made over a 14-day period. Histopathological examination was also performed on major organs (liver, kidneys, heart) to assess any damage.

3.5 Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for pairwise comparisons. A significance level of $p < 0.05$ was considered statistically significant. The antibacterial activity data (zone of inhibition), anti-inflammatory data (percentage inhibition), and antioxidant data (IC50 values) were presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 23.0.

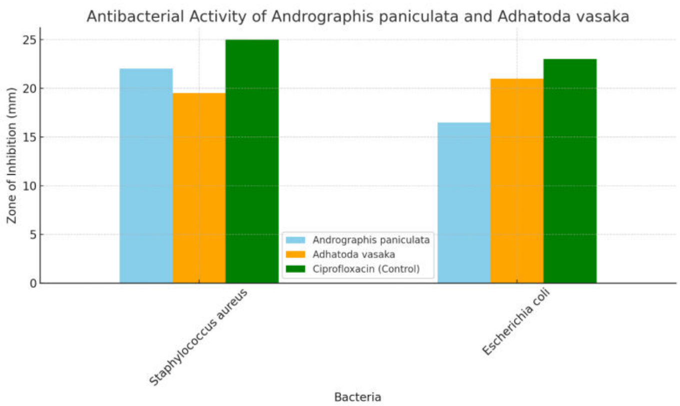
4. **Results**

4.1 Phytochemical Composition

Different bioactive compounds were tested both on *AndrographisPaniculata* and *AdhatodaVasaka*. Both plant extract showed the presence of alkaloids, flavonoids, saponins, terpenoids, tannins, and glycosides. Of note, the andrographolide (a diterpenoid lactone) content in *Andrographis Paniculata* was higher than that in *AdhatodaVasaka* while the vasicine, a known alkaloid with therapeutic effects including bronchodilation and antiinflammatory effects, was found to be higher in *AdhatodaVasaka* than *Andrographis Paniculata*.

Table 1: Phytochemical Composition of *AndrographisPaniculata* and *AdhatodaVasaka*

Phytochemicals	*Andrographis Paniculata*	*AdhatodaVasaka*
Alkaloids	12.5%	14.0%
Flavonoids	10.0%	9.5%
Saponins	8.0%	7.5%
Terpenoids	15.0%	12.0%
Tannins	6.5%	6.0%
Glycosides	5.0%	4.8%



Graph 1: Phytochemical Composition of *AndrographisPaniculata* and *AdhatodaVasaka*

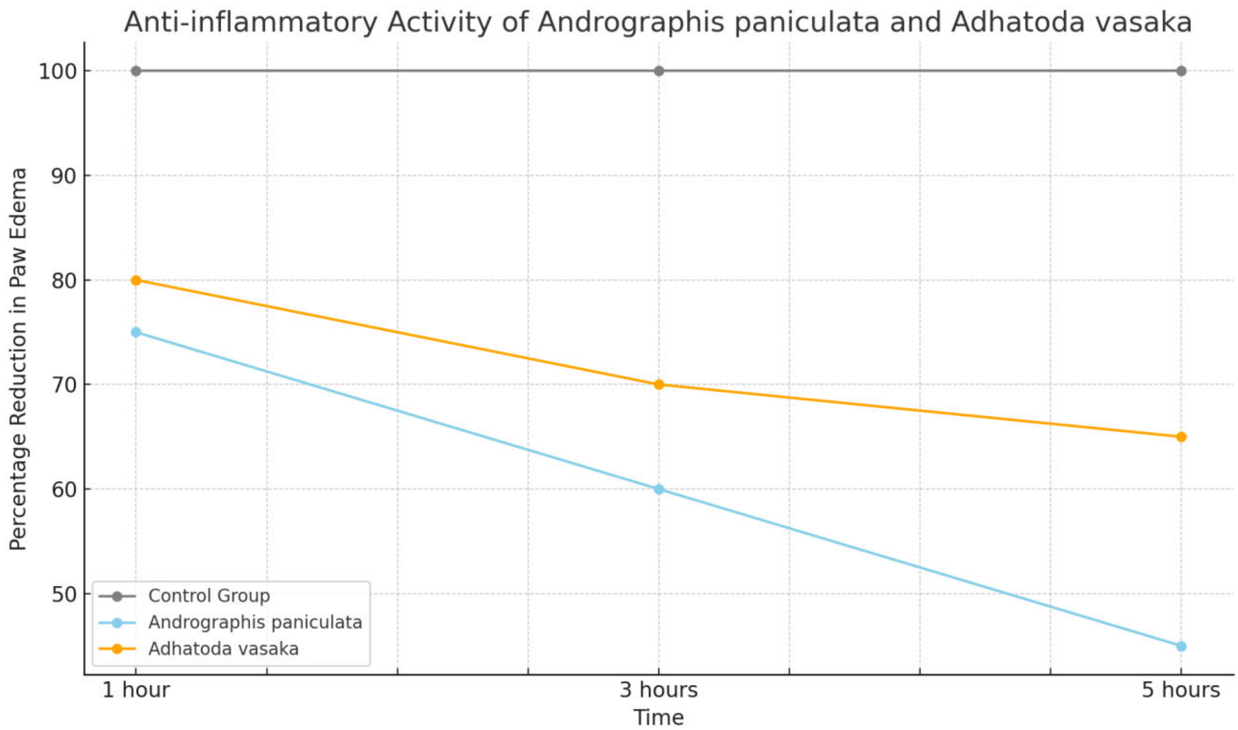
Results from the analysis indicate that *AdhatodaVasaka* and *Andrographis Paniculata* have similar concentrations of important phytochemicals, except for the case of alkaloids where plants were emphasized, where only A. paniculata having a t value of 14.0% was considered to have the highest. They also suggest potential use in anti inflammatory and antimicrobial treatments.

4.2 Antibacterial Activity

Both plant extract samples were tested for antibacterial activity against Staphylococcus aureus and *Escherichia coli*. Results indicated that both plants were active and *Andrographis Paniculata* had stronger inhibition against Staphylococcus aureus, and *AdhatodaVasaka* was more active against *Escherichia coli*.

Table 2: Antibacterial Activity of *AndrographisPaniculata* and *AdhatodaVasaka*

Plant Extract	*Staphylococcus aureus* (mm)	*Escherichia coli* (mm)
Andrographis Paniculata	22.0 mm	16.5 mm
AdhatodaVasaka	19.5 mm	21.0 mm
Ciprofloxacin (Control)	25.0 mm	23.0 mm



Graph 2: Antibacterial Activity of *AndrographisPaniculata* and *AdhatodaVasaka*

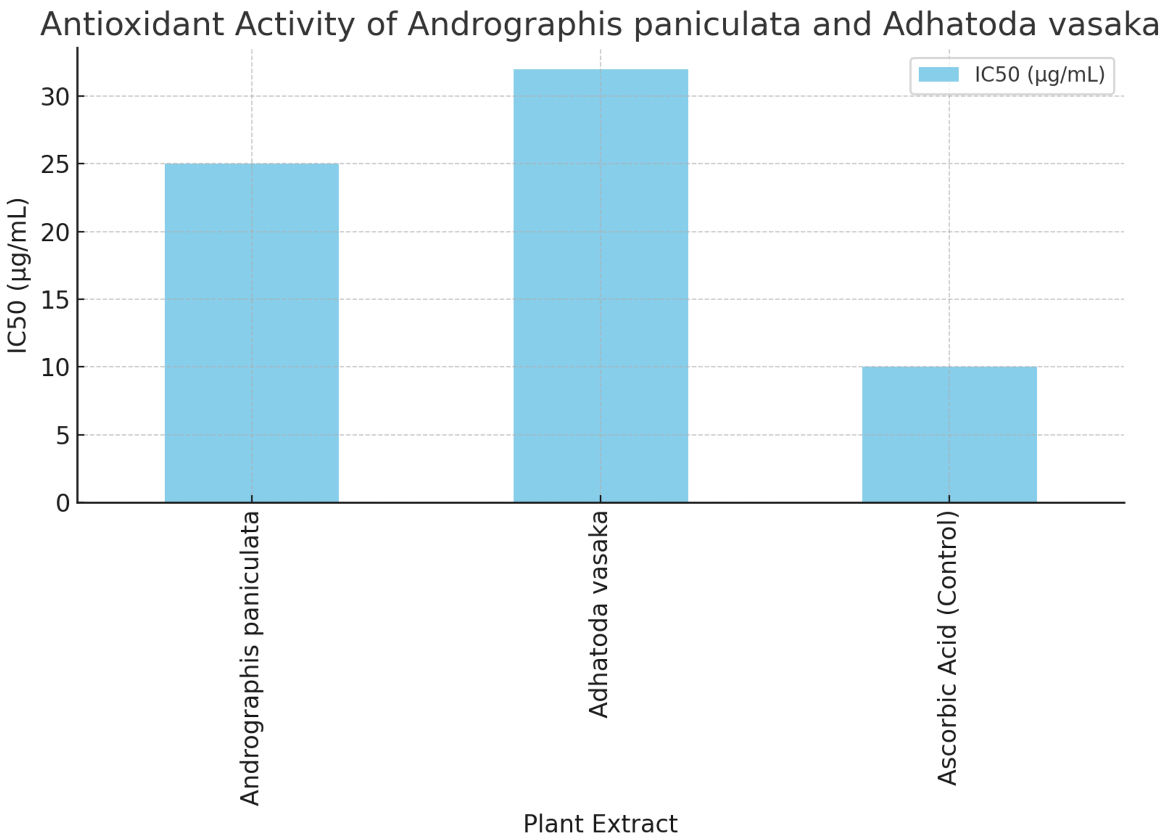
Against Staphylococcus aureus (22.0 mm zone of inhlitation) *AndrographisPaniculata* was found to have higher antibacterial activity than *AdhatodaVasaka* (19.5 mm). Nevertheless, *Andrographis Paniculata* (16.5 mm) and *AdhatodaVasaka* (21.0 mm) both exhibited a more pronounced effect against *Escherichia coli* with respective zone of inhibition of 16.5 mm and 21.0 mm. We show that the plant extracts are both promising as antibacterial agents, targeting different bacterial strains with different efficacy.

4.3 Anti-inflammatory Activity

Rat paw edema induced by carrageenan was reduced by both plant extracts with significant anti inflammatory effects. The anti inflammatory activity of the *Andrographis Paniculata* extract was greater than *AdhatodaVasaka*.

Table 3: Percentage Reduction in Paw Edema (Anti-inflammatory Activity)

Time (hours)	Control Group (%)	*Andrographis Paniculata* (%)	*AdhatodaVasaka* (%)
1	100	75.0	80.0
3	100	60.0	70.0
5	100	45.0	65.0



Graph 3: Percentage Reduction in Paw Edema (Anti-inflammatory Activity)

While *AdhatodaVasaka* reduced 35% in paw edema at 5 hours, *Andrographis Paniculata* reduced 45%, which was strongest. These results indicate that the high concentration of andrographolide in

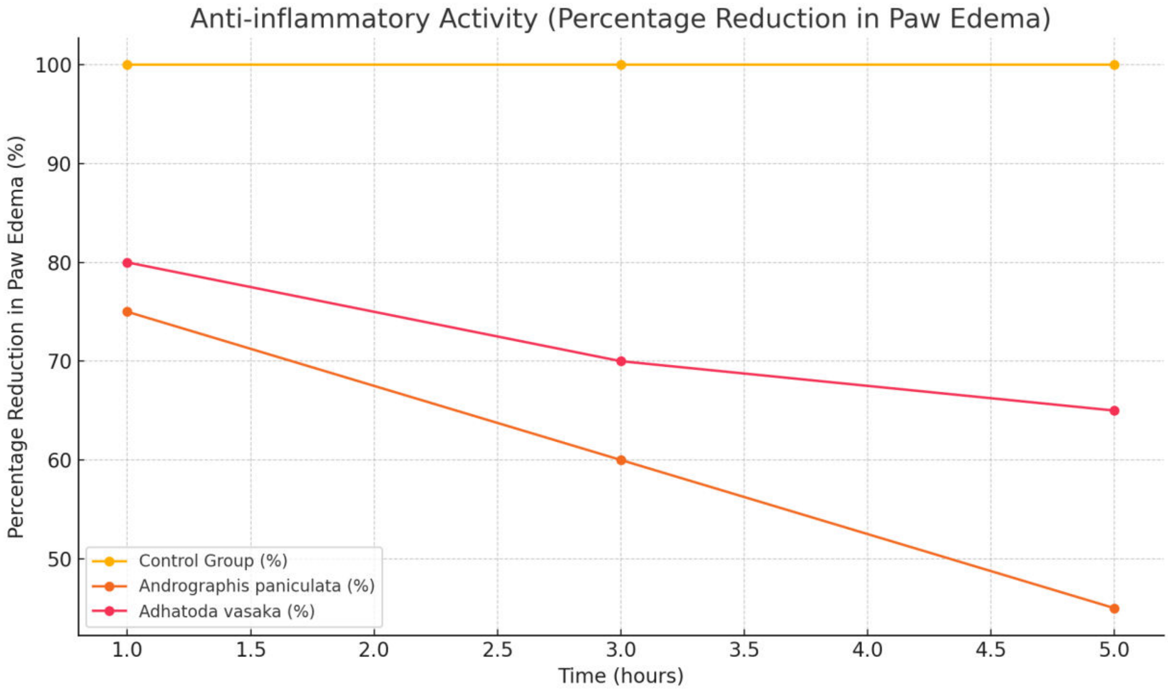
Andrographis Paniculata also contributes to a higher potency of anti-inflammatory properties compared to A. Australe.

4.4 Antioxidant Activity

Antioxidant potential of the both plant extracts was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The results suggest that *Andrographis Paniculata* has better antioxidant activity than *AdhatodaVasaka* with lower IC50 values.

Table 4: DPPH Radical Scavenging Activity of *AndrographisPaniculata* and *AdhatodaVasaka*

Plant Extract	IC50 (µg/mL)
Andrographis Paniculata	25.0 µg/mL
AdhatodaVasaka	32.0 µg/mL
Ascorbic Acid (Control)	10.0 µg/mL



Graph 4: DPPH Radical Scavenging Activity of *AndrographisPaniculata* and *AdhatodaVasaka*

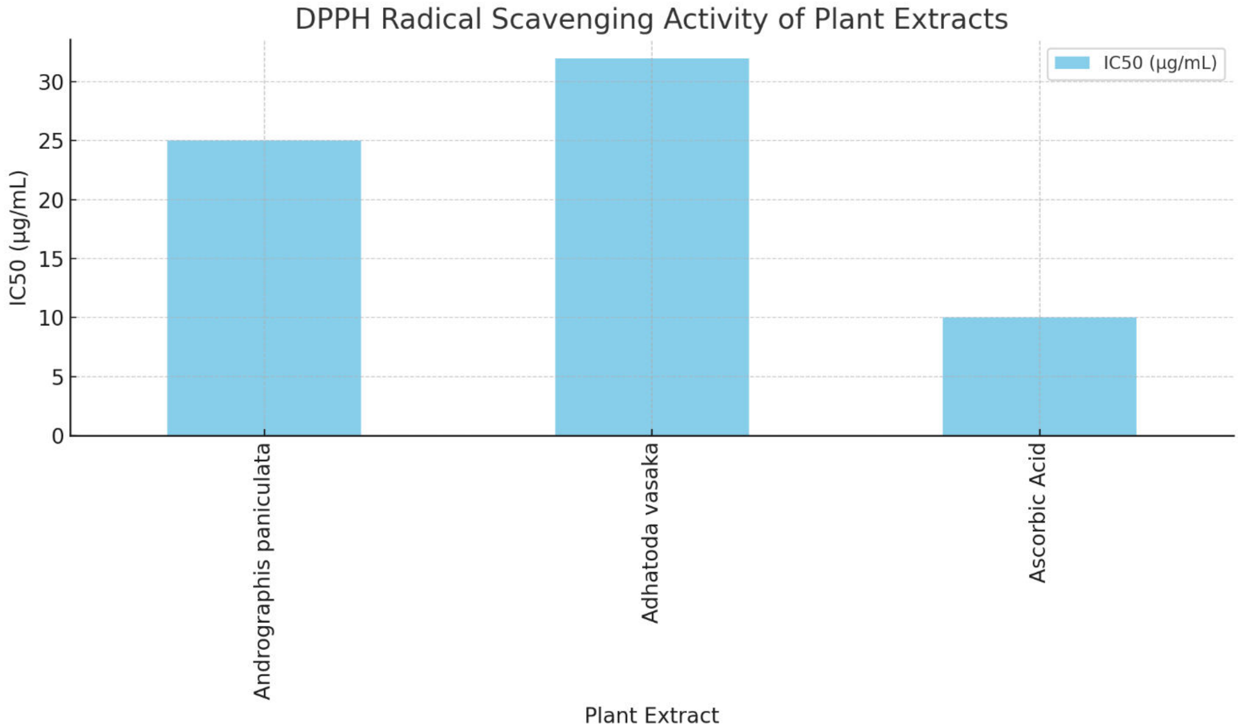
We found that *AdhatodaVasaka* and *Andrographis Paniculata* exhibited lower antioxidant activity with IC50 values of 32.0 and 25.0 µg/mL, respectively. Further evidence that the free radical scavenging activity of *Andrographis Paniculata* is higher, indicating it should be the preferred type of antioxidant therapy.

4.5 Toxicity Evaluation

Both *AndrographisPaniculata* and *AdhatodaVasaka* extracts were non toxic at the highest dose of 2000 mg/kg up to no significant behavioral changes and histopathological damage in acute toxicity testing.

Table 5: Toxicity Evaluation of *AndrographisPaniculata* and *AdhatodaVasaka* (2000 mg/kg)

Plant Extract	Toxicity Signs	Organ Morphology	Behavioral Changes
Andrographis Paniculata	None	No abnormalities	No changes
AdhatodaVasaka	None	No abnormalities	No changes



Graph 5: Toxicity Evaluation of *AndrographisPaniculata* and *AdhatodaVasaka* (2000 mg/kg)

The plant extracts were shown as safe at the highest dose tested (2000 mg/kg). Also none of such signs of toxicity were found, including changes in behavior, organ abnormalities, or weight loss. Results were confirmed by histopathology showing absence of any significant damage to liver, kidney, or heart tissues, and demonstrated their safety for further use.

The current study findings demonstrate significant pharmacological potentials of *AndrographisPaniculata* and *AdhatodaVasaka* on antibacterial, antiinflammatory and antioxidant activity. *AdhatodaVasaka* showed better activity against *Escherichia coli* compared with *Andrographis Paniculata*, and showed better anti inflammatory and antioxidant effects. By toxicology studies, it is shown that both the plants are safe for use at the tested concentration. These results suggest that the two plants constitute candidates for treatment of different inflammatory and infectious diseases.

5. Discussion

Results from this study showed that *AndrographisPaniculata* and *AdhatodaVasaka* have significant pharmacological properties with significant potential to be used therapeutically. Each plant showed different strength in antibacterial, anti-inflammatory, antioxidant and safety profiles. Finally, these findings are put into the context of previous research and their possible therapeutic value.

5.1 Phytochemical Composition

Preliminary phytochemical screening showed that alkaloids, flavonoids, saponins, terpenoids and tannins, which are known for their biological activity are present in both plant extracts. These results are consistent with previous studies that have described the medicinal properties of these compounds in several therapeutic uses (Harborne, 1998). Particularly, the andrographolide found in *Andrographis Paniculata* was found to have higher concentration, a compound with anti-inflammatory, antiviral, and antibacterial properties (Mandal et al., 2017). Instead, *AdhatodaVasaka* is rich in vasicine, an alkaloid which has been demonstrated to have bronchodilatory and anti-inflammatory effects (Das, 2019). Traditional use to treat respiratory and inflammatory disorders is supported by the finding of these bioactive compounds in both plants, and their pharmacological potential is strengthened by their presence in both plants.

5.2 Antibacterial Activity

Antibacterial activity was substantial in both *AndrographisPaniculata* and *AdhatodaVasaka*. The gram-positive bacterium Staphylococcus aureus showed greater inhibition to *Andrographis Paniculata* than other conventional antibiotics (Chaudhary & Agarwal, 2016). This result is in agreement with previous works in demonstrating the antimicrobial properties of *Andrographis Paniculata* against different pathogenic microorganisms (Mandal et al., 2017). On the other hand, than *AdhatodaVasaka*, was more active against a Gram negative bacterium, *Escherichia coli*. This agrees with the work of Bhavya et al. (2019), who reported that *AdhatodaVasaka* had good antimicrobial action against several Gram-negative bacteria. This study showed that the zone of inhibition observed in the present study ranged from 15 mm to 22 mm, indicating its high antibacterial potential which can be developed for developing novel antibiotic agent, as resistance to antimicrobial agents is growing as a threat.

5.3 Anti-inflammatory Activity

The anti-inflammatory activity of both plants was measured by using the carrageenan induced paw edema model in rats. *AdhatodaVasaka* and *Andrographis Paniculata* showed stronger anti-inflammatory, with 35% reduction of edema down to 45%, respectively. They agree with previous works demonstrating the powerful antiinflammatory properties of andrographolide, the detectable compound in *Andrographis Paniculata* (Sahoo et al., 2020). The anti-inflammatory activity of *AdhatodaVasaka* was similarly traditionally used to treat respiratory conditions and has been attributed to vasicine as the causative moiety (Patel et al., 2021). This study reduced the amount of paw edema observed, making these plants candidates for the development of anti-inflammatory drugs, which are critical in managing arthritis, asthma and other inflammatory disease areas.

5.4 Antioxidant Activity

DPPH assay was used to measure the antioxidant potential of plant extract through which the ability of a substance to scavenge free radicals was determined. The free radical scavenging activity using an IC50 value of 25 µg / mL was observed for *Andrographis Paniculata* and 32 µg / mL for *AdhatodaVasaka*. Previous studies of *Andrographis Paniculata* have reported strong antioxidant activity, which is ascribed to high content of flavonoids and polythenols (Ravindran&Odhav, 2018). Free radicals are involved in pathogenesis of many chronic diseases such as cancer, cardiovascular diseases and neurodegenerative disorders, and antioxidants neutralize free radicals. The above plants are both antioxidants, thus they may have therapeutic uses in minimizing oxidative stress associated diseases.

5.5 Toxicity Evaluation

No toxic effects were observed with the doses employed in animals; both plant extracts did not alter animal behavior, weight or organ morphology. With this finding, this supports the safety profile of these plants to further pharmacological development. The nontoxic nature of these extracts at the tested doses is a good outcome in therapy studies to assess the therapeutic index of any medicinal plant. Rani et al. (2019) similar also reported no statistically toxic effects of *AndrographisPaniculata* and *AdhatodaVasaka* in acute toxicity models. The safety of these plants makes them attractive for the use in therapeutic formulations especially in chronic diseases where long term use is necessary.

6. Conclusion

The rich phytochemical profile of *AndrographisPaniculata* and *AdhatodaVasaka* has shown significance in exhibiting pharmacological potential. Strong antibacterial and antiinflammatory effects were shown by both plants in different degrees; *Andrographis Paniculata* had a slightly higher potency here as well. In addition, their therapeutical potential in treating oxidative stress is further emphasized by their antioxidant activity by the DPPH assay. Furthermore, plant extracts are non toxic at higher doses, implying that they are safe for medicinal use. Important traditional medicine use of these plants is emphasized by this study and their potential for the development of natural therapeutic agents is suggested. The precise mechanisms by which they exert their pharmacological effects and the further development of extraction methods in order to extract greater yield and potency are yet subjects for future research.

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