Research Article

Phytochemical analysis and antimicrobial activity of *adhatoda vasica* leaves

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Abstract

The experiment was conducted to estimate the phytochemical and antibacterial activity in *Adhatoda vasica* during the year 2018. The plant samples were collected from the Khanpur valley in fresh forms and were processed in the Food Science and Technology Laboratory The University of Haripur for further chemical analysis. Antimicrobial activity of *Adhatodavasica* was tested against various gram positive and gram negative bacterial strains. Different phytochemicals compounds i.e. Alkaloids, Steroids, Flavonoids, Terpenoids, Tannins Extract, Saponins, and Glycosides were investigated in the experiment. Highest antimicrobial activity was recorded in case of gram positive bacteria. Leaf extracts of *Adhatodavasica* leaves extract would be replacement to synthetic/artificial antimicrobial agents and needs further studies and clinical trials. The phytochemical screening indicated presence of secondary metabolites such as alkaloid, flavonoids, tannins and phenol in both extracts making it to have antibacterial potentials. Extract showed remarkable activity against the growth of the selected bacteria The presence of these phytochemicals may lead to the conclusion that *Adhatodavasica* plants can be used as medicine for treating different diseases.

Keywords: *Adhatodavasica*; Antibacterial activity; *Escherichia coli*; Phytochemicals; *Staphylococcus aureus*

Introduction

Adhatoda vasica commonly known as Vasaka, Baker or Malabar Nut belongs to Acanthaceae family [1]. It is evergreen plant (1.0 m to 2.5 m height) having bitter taste and unplesent smell. It has approximately height of 1 to 2.5 m [2]. The leaves and flowers of this plant are extensively used for curing asthma, cough, cold, expectorant and antispasmodic. *A.Vasica* reported to prevent oxidative

damage of carbon tetrachloride induced hepatotoxic effect in rats. [3] accounted the highest amount of phenolic compounds with scavenges the free radicals and exhibits greatest antioxidant activity. The positive effect of gamma irradiation on the natural antioxidants of *A.Vasica* showed release of phenolic compounds [4]. The ethanolic extract of *A. vasica* showed high antioxidant activity with cytoprotective potential in cell culture [5]. Interestingly,

the methanolic and aqueous extract of *A. vasica* has potential phytochemical composition of flavonoids, phenols with antioxidant and cytotoxic effect [6]. Recently, [7] evaluated pharmacological screening of leaf extract of *A. vasica* against dysentery and diarrhea due to presence of chemical compounds tannins, alkaloids, saponins and flavonoids.

Many plant species possess antioxidant effects and can be used for medicinal purposes. *Adhatoda* is the species widely distributed throughout the world. This species is available in Pakistan and particularly in Khyber Pakhtunkhwa region [8]. Hence chemical analysis of *Adhatoda vasica* is of immense interest to validate its use in traditional medicine for the cure of many ailments and to find its active principles by characterization and isolation of its components. The screening of different phytochemicals of *Adhatodavasica* can help in detection of new active compounds.

Materials and methods

The present study was conducted in Food Science and Technology laboratory, The University of Haripur, Khyber Pakhtunkhwa, Pakistan during 2018 to find out the antimicrobial activity of *Adhatoda vasica* along with its phytochemical analysis properties. Fresh leaves of *Adhatoda vasica* were collected from Khanpur valley of District Haripur, Hazara. Leaves were carried to Food Science and Technology Laboratory for analysis. The leaves were washed thoroughly with tap water in order to remove any dust and other unwanted particules. Leaves were then air dried for 25 days at room temperature and then grounded with an electric grinder to obtain fine powder. About 100 g of powdered leaves were transferred into round bottom flask and 750 mL ethanol & 250 mL chloroform were added respectively and placed in dark for 72hrs with occasional stirring. The extract was filtered with Whatman filter paper no 4. The filtrates were then passed through rotary

evaporator at a temperature of 45° C followed by drying in a desiccator. The gel like crude extracts were stored in refrigerator for further analysis.

Analysis of phytochemical

Leaf extracts of *Adhatodavasica* were then subjected to regular phytochemical analyses to uncover the presence or absence of following phytochemicals alkaloids, steroids, flavonoids, Terpenoids, Tannins Extract, Saponins and Glycosides [9-11].

Antibacterial activity assay

Anti-microbial activity of *Adhatodavasica* was assessed by using Disc Diffusion Method [12]. Four different bacterial strains were collected from Microbiology Lab, The University of Haripur. Pure cultures were sub cultured in Muller Hinton agar as suggested by [13]. The plates were poured with 15ml of Müller-Hinton agar. These plates were kept for 5 min to solidify. *Adhatodavasica* extracts were then loaded on 5mm sterile individual disc. These loaded discs were then positioned on top of medium and allowed to diffuse with each other for 5min. Later on these plates were kept in incubator at 37°C for 24h. Same solvent was used for preparation of negative control whereas positive control was gentamycin (10 µg/disc). Incubation zones were measured at the end of the incubation using transparent ruler in millimeters.

Results and discussion

Phytochemical analysis

The leaf extracts (ethanol, chloroform) of *A. vasica* have been analyzed for the presence of qualitative phytochemicals. Qualitative phytochemical analysis based on the methods described by [14]. During the analysis, these extracts were analyzed for Steroids, Tannin, Saponin, Anthocyanin, Coumarins, Alkaloids, Proteins, Amino acids, Diterpenes, Phytosterol, Phenol, Leucoanthocyanin, Cardiac glycosides and Flavonoids (Table 1). It can be observed from (Fig. 1) that chloroform extracts were unable to show

the presence of alkaloids while ethanol was proved to be the suitable medium for extraction of alkaloids from *A. vasica* leaves. Alkaloids are proven to cause the beneficial biological compound for human health and is important part of diet for ages. Recently, it is used as supplements and pharmaceuticals and in other applications in human life. Moreover, they showed the potential for organic synthesis for searching new semisynthetic and synthetic compounds with possibly better biological activity in controlling and preventing many disease. than parent compounds [15]. From (Fig. 2), it is observed that ethanolic extraction method failed to extract steroids from *A. Vasica* leaves while chloroform as solvent proved to extract these phytosterols may also act as precursors for the de novo biosynthesis of steroid hormones [16]. Flavonoids, terpenoids and saponins (Fig. 3, 4 & 5) were present in both extracts which shows promising capabilities of both extracts for prevention and treatment of many degenerative diseases. Tannins are water soluble compounds which is an important raw material for sustainable green industries such as leather, feed, fisheries, beverages, etc [17]. (Fig. 6) showed that these water soluble compounds can be extracted by using chloroform as a solvent while ethanol has been showed no efficacy in extraction. Glycosides (Cardiac glycoside) are important for increasing the output force of the heart and increase

its rate of contractions by acting on the cellular sodium-potassium ATPase pump [18] which were present in ethanolic extracts while chloroform was unable to extract these (Fig. 7) showed presence in ethanolic extracts. The results also showed the antibacterial activity of these leaf extracts of *Adhatodavastica* against various pathogens (Fig. 8, 9 & 10). Tannins are well documented to exhibit the biologically active compounds against *E. coli* and *S. aureus* [19]. Results obtained from this study indicates that by using agar-well diffusion method, pure ethanolic extracts of *A. vasica* leaves showed maximum antimicrobial effects against the tested bacteria. Therefore current study also provided a justification of using ethanolic extraction method to obtain phytochemicals which can sway the disease causing pathogens [20]. Same results were also observed by earlier researchers, who noted that major portion of medicinal plants have least effectiveness against gram negative as compared to gram positive organisms [21]. Chloroform and ethanolic extracts of *A. vasica* leaves have a potential antibacterial activity against bacterial pathogens and to cure infections caused by bacterial such as *E. coli*, *S. aureus* and *S. pyogenes*. Nevertheless, the result in this study could also serve as a basis for more investigations on the plant, *A. vasica* and its antimicrobial capabilities.

Table 1. Phytochemical analysis of *A. vasica* leaves

S. No.	Phytochemicals	Chloroform Extract [+ means Present; - means Absent]	Ethanol Extract [+ means Present; - means Absent]
1	Alkaloids		
	Wagner's	-	+
2	Steroids	+	-
3	Flavonoids	+	+
4	Terpenoids		
	Salkowski	+	+
5	Tannins Extract	+	-
6	Saponins	+	+
7	Glycosides		
	Cardiac glycoside (Keller-Killiani test)	-	+

Figure 1. Alkaloids Wagner's Test of (a) chloroform (Negative) and (b) ethanolic (Positive) extracts

Figure 2. Steroid Test of (a) chloroform (Positive) and (b) ethanolic (Negative) extracts

Figure 3. Flavonoid Test of (a) chloroform (Positive) and (b) ethanolic (Positive) extracts

Figure 4. Terpenoids(Phtosterol) Salkowski's Test of (a) chloroform (Positive) and (b) ethanolic (Positive) extracts

Positive

Positive

Figure 5. Saponin Test of (a) chloroform (Positive) and (b) ethanolic (Positive) extracts

Figure 6. Tanins Test of (a) chloroform (Positive) and (b) ethanolic (Negative) extracts

Negative

Positive

Figure 7. Cardiac glycoside Test of (a) chloroform (Negative) and (b) ethanolic (Positive) extracts

Figure 8. Antibacterial activity of chloroform and ethanolic extracts of *A. Vasica* leaves

Figure 9. Antibacterial zone of chloroform and ethanolic extracts of *A. Vasica* leaves against *E. coli* (L) and *S. aureus* (R)

Figure 10. Antibacterial zone of chloroform and ethanolic extracts of *A. Vasica* leaves against *P. aureus* (L) and *S. pyogenes* (R)

Conclusion

The current study was aimed at the evaluation of *A. vasica* leaf extracts for phytochemical profiling along with their antibacterial potential. The results showed promising indication of presence of phytochemicals like steroids, flavonoids, terpenoids, tannins and Saponins except alkaloids and cardiac glycosides in the chloroform extracts. While lacks in steroids and terpenoids. Both of the extracts showed antibacterial activity against *E. coli*, *S. aureus* and *S. pyogenes*, however, ethanolic extracts exhibited maximum activity against *E. coli* and *S. aureus* and can be used in various food, pharmaceutical and cosmetic products.

Authors' contributions

Conceived and designed the experiments: Q Shahzad & S Sammi, Performed the experiments: Q Shahzad & Q Ayub, Analyzed the data: A Mehmood & M Hussain, Contributed materials/ analysis/ tools: K Naveed, K Azeem, M Hassaan & A Ayub, Wrote the paper: A Mehmood & O Shokat.

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