**ABSTRACT:** Pharmacological research has shown interest in various alkaloidal molecules. A new approach for enhancing the bioactivity of a molecule can be through a structural modification in the phytochemical. Present investigation involves the treatment of alkaldoids from *Adathoda vasica*, using *Aspergillus niger* producing pectinase enzyme. The structural modification after a period of one week was studied using HPTLC. A change in the HPTLC profile was observed indicating a change in molecule. The new transformed molecule showed an Rf value of 0.06 as compared to the Rf value of 0.50 for the untreated alkaloid control. The transformed molecule exhibited a significant increase in antioxidant activity determined by the Potassium ferricyanide reducing antioxidant power (PFRAP) method. The results prove the biotransformation of the alkaloid using *Aspergillus niger* producing pectinase enzyme.

**Key words:** alkaloids, biotransformation, Pectinase, *Adathoda*.

**INTRODUCTION:**

Alkaloids are heterogeneous group of basic nitrogen containing substances found in higher plants. Vasicine is a quinazoline type of alkaloid which is mainly obtained from the plant *Adathoda vasica* (Rachana et al., 2011). *Adhathoda vasica* is a primary herb of the ayurvedic system used in the treatment of coughs, bronchitis, asthma and symptoms of common cold (Sampath Kumar et al., 2010). It is used as an ingredient in numerous popular formulations, including cough syrups, in which it is frequently combined with tulsi (holy basil) and ginger. Its main action is as an expectorant and antispasmodic (bronchodilator) (Singh et al., 2011). Its important active components include alkaloids vasicine and vasicinone (Karthikeyan et al., 2009). Typically herbal formulae are composed of multiple herbs, used in combination, so as to produce the desired therapeutic effect and reduce toxic side effects. However most bioactive compounds derived from plants are metabolic derivatives of phytochemicals, due to which it has been harder to demonstrate and to correlate the efficacy of these phytochemicals in vitro (Lui and Henkel, 2002).

Transformation of the pro-drug phytochemicals obtained from plants can be carried out by physical, chemical and biological methods. Biotransformation using microorganisms and/or isolated enzymes has been increasingly exploited in both academic studies and industries. Biotransformation has a vast range of advantages over physical and chemical transformations. Whole cell biotransformation utilizes whole microorganisms along with substrate in a bulk system. The advantages of whole cell biotransformation over isolated enzymes are, higher activity of enzyme is obtained in growing cultures, co-factor recycling is not required, less cost input, and easy maintenance (Loughlin, 2000). The therapeutic efficacy of the biotransformed phytochemicals can be established by studying their antimicrobial action and their antioxidant properties. The biotransformed phytochemical(s) may have significantly enhanced bioactivity, thereby increasing the potential applications of their herbal formulations.

**MATERIALS AND METHODS:**

The plant material of *Adathoda vasica* was obtained from Konark herbal and health care. 100gm of plant material was then dried, crushed, powdered, and was used for the study. *Aspergillus niger* producing pectinase was isolated and identified by molecular methods and maintained using pectin agar.

Microbial transformation was carried out using pectinase producing *A. niger*. The microbial transformation was done using fermentation broth consisting of 10 g of plant material, 0.1% peptone, 0.5% yeast extract, 0.01% KH₂PO₄ and 0.1% NaCl. 48 hour old culture of *A. niger* producing pectinase was inoculated and transformation was allowed for a period of one week under shaker conditions (Barredo, 2005). Uninoculated broth containing plant material was maintained as control.

After a period of one week, the crude alkaloids were extracted from treated and untreated control flasks. The procedure involved extracting alkaloids from the filtered and dried residue (both treated and untreated) with 10% acetic acid and methanol, leaving it to stand for at least 4 hours. The extract was then concentrated to one quarter of the original volume and the alkaloids were precipitated by drop wise addition of concentrated ammonia solution. The alkaloids were collected by centrifugation, and washing with 1% ammonia solution. The alkaloids extracted were reconstituted in distilled water and used for further analysis (Harborne, 1998).

The antioxidant activity of the crude extracts was determined by estimation of Fe⁺ reducing power by PFRAP method (Hajaji et al., 2010). The intensity of iron (II) ferricyanide complex was determined by measuring the formation of Perl's Prussian blue. Gallic acid was used as positive control for the reaction.

High performance Thin Layer Chromatography (HPTLC) analysis of the untransformed control and one week old transformed alkaloids extracted was carried out at Anchorme India (PVT) Mumbai. The sample was loaded on precoated silica gel plates and developed using toluene: ammonia (1: 9). The comparative TLC profile of transformed alkaloid and control alkaloid extracts were scanned and documented.
RESULTS AND DISCUSSIONS:

The extracts obtained were analyzed by HPTLC to prove the expected structural modification of the transformed alkaloids. The HPTLC profile of the control untreated alkaloid showed a single peak with an Rf value of 0.50 (figure 1 & 3). However the transformed alkaloid showed a new peak with an Rf of 0.06 (figure 2 & 3). The antioxidant activity of the treated alkaloid extract was significantly higher than the untreated alkaloid (control) determined by the Potassium ferricyanide reducing antioxidant power (PFRAP) method (figure 4). The results confirm the antioxidant activity of the extracts proven through various studies (Srinivasarao et al. 2006; Ilango et al. 2009). However, the higher antioxidant activity of the biotransformed alkaloid may be as a result of various enzymatic modifications such as hydroxylation, and reduction of the present alkaloid by the *A. niger*. Also, the study utilizes aqueous system over organic solvents for biotransformation and activity analysis due the widespread applications of water soluble pharmacologically active constituents in herbal medicine.

**Figure (1) HPTLC profile of control extract**

![HPTLC profile of control extract](image1)

**Figure (2) HPTLC profile of transformed extract.**

![HPTLC profile of transformed extract](image2)

**Figure (3) HPTLC plate after derivatization (at 366nm)**

![HPTLC plate after derivatization](image3)

Lane 1- control-alkaloid extract
Lane 2- transformed alkaloid

**Figure (4) Comparison of the Antioxidant activity of the control alkaloid extract and the transformed alkaloid extract.**

![Antioxidant activity comparison](image4)

CONCLUSION

The result of the present study proves that microbially transformed alkaloid from *Adathoda vasica*, after transformation by *A. niger*, shows comparatively higher antioxidant activity as compared to untreated control. The change in the HPTLC profile of the transformed extracts confirms the chemical modification in the pharmacologically active transformed molecule. Thus microbial transformation has resulted in significant alteration of structural and antioxidant function of the alkaloids present, enhancing its overall potential applications. Such bioactive transformed molecules hold a key in the pharmacological studies of medicinal plants.

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