Trends in Life Sciences
An International Peer-reviewed Journal
www.sciencejournal.in

# CHARACTERIZATION OF ALKALOID PRODUCED BY *ASPERGILLUS* SP STRAING TAS1: IT'S POSSIBLE ROLE AS ANTIOXIDANT AND ANTIBACTERIAL AGENT

**Smriti Sharma[1] and Trupti K Vyas*[2]**
[1]Quality Control Division, Claris LifeScience Ltd, Charcharwadi, Vasna, Changodara, Gujarat, India,
[2] Food Quality Testing Laboratory, Navsari Agricultural University, Navsari – 396450, Gujarat, India
**Corresponding author :** Email –vyastrupti@hotmail.com

ABSTRACT
Alkaloid production by *Aspergillus* sp strain TAS1 was examined. Alkaloid was characterized by TLC, FTIR and UV-Vis spectroscopy. Data revealed that purple color band on thin layer chromatogram, characteristics bond by IR spectroscopy and peaks by UV-Vis confirm the alkaloid production by isolate. Effect of addition of tryptophan on alkaloid production indicated the synthesis was increased almost two fold upon its addition. Moreover, temporal effect on production revealed that production was higher on 20$^{th}$ day of incubation, thereafter decreased. Alkaloid inhibits growth of *P. aurogenosa*, *E. coli*, and *S. aureus*. ABTS assay showed alkaloid has 95.5 % antioxidant activity. Hence, it can be used as potential antioxidant agent.

**KEYWORDS:** alkaloid, antioxidant, antibacterial, *Aspergillus*

## INTRODUCTION

Microorganisms especially fungi provide us with an enormous variety of secondary metabolites. During their interaction with plant these fungal isolates produce structurally unique and potential bioactive compounds. Alkaloids are physiologically active, nitrogen containing secondary metabolites with low molecular weights produced by many fungi. Ergot is well known alkaloid produced by grass associated fungi. The ergot alkaloids are so named because they are made by ergot fungi; that is, *Claviceps* species. *Claviceps* produced hard resting structure known as Ergots during infection to grass. Screening analyses of other fungi for ergot alkaloids have identified several distantly related fungi as potential sources (Flieger *et al*, 1997; Kozlovsky, 1999; Spilsbury and Wilkinson, 1961). *Aspergillus* spp are most explored for the alkaloids production and some of which have antifungal, antibacterial, anti-HIV and cyto-toxic activity.

Living cells produced reactive oxygen species during their metabolic processes. Such ROS damage biomolecules like protein, DNA and lipid leading ultimately cell damage. Antioxidants compounds scavenge free radical molecules and prevent from cellular damage. Chemically derived antioxidant use to prevent free radical damage has been reported to have toxic side effect (Radulovic *et al*, 2007). Hence, exploration for new natural antioxidants and free radical scavengers is required. Consequently, present study aimed towards finding novel antioxidant from natural resources. Here antioxidant potential of alklaloid produced by fungal isolate was examined.

## MATERIALS AND METHODS

### Isolation and identification:

A fungus was isolated from the soil sample on potato dextrose agar and purified. Isolate was stored on PDA at 4 °C until the use. Isolate was identified on the basis of its colonial and morphological characteristics by microscopy.

### Screening for alkaloid production and its characterization:

To verify the alkaloids production, fungal culture (7mm diameter from 4 days grown ceulture on PDA) was inoculated in 100 ml medium (g/L; sorbitol, 100g; glucose, 40g; succinic acid, 10g; KH2 PO4 , 1.0g; MgSO 4 .7H2 O, 0.3g; yeast extract, 1.0g; FeSO 4 .7H2 O, 0.1mg; CuSO 4 . 5H2 O, 0.01mg ; ZnSO 4 . 7HzO, 0.01mg ; MnSO 4 .H2 O 0.001mg. pH 5.2) in 250 ml Erlenmeyer flask. Flask was incubated on shaker at 150 rpm at room temperature for 20 days. Alkaloid production was examined by Mayer's test (Evans, 2005) Dragendorff's test (Waldi, 1965) Hager's test (Wagner *et al*, 1996) and Wagner's test (Wagner, 1993) were perform to confirm the presence of alkaloid production. After incubation period, 10ml filtrate was neutralized and extracted with chloroform and residue was subjected for TLC (Kozlovsky *et al*, 2000) and FTIR analysis (4000-400 cm-1 range (Nicolet IR200 FT-IR Spectrometer).

  

**Temporal effect:**
To observe the temporal effect on alkaloid production, fungus was grown in media as mentioned above and incubated for 20 days. After every four days of interval, sample was withdrawn for alkaloid production profile. Sample was analyzed after extraction, by UV-Vis spectroscopic scanning between 200 – 500 nm in spectrophotometer.

**Effect of treptophan on alkaloid production :**
Addition of tryptophan increase alkaloid production (Vining, 1970; Camilo *et al*, 1998). A fungus was grown in media supplemented with tryptophan (0.5µg/ml) to examine its effect. Alkaloid was extracted from media after 20 day of incubation and production was analyzed by UV-Vis scanning.

**Antimicrobial and antioxidant activity :**

Antimicrobial activity of the alkaloids was evaluated against three different culture viz. *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The Antibiotic Tertracyclin and Ampicillin were used as standard. Antioxidant activity was measure by ABTS assay (Re *et al*, 1999). Absorbance was measured at 734 nm exactly 1 minute after initial mixing and up to 6 minutes. Ascorbic acid was used as standard.

**RESULTS**
Five different fungus cultures from soil sample grown on PDA plates were selected and purified further. These five cultures were selected on the basis of their different colony morphology. Among these five, 2 were *Aspegillus* sp. produced black and green colony; one *Penicillium* sp. produced green color colony with yellow color on back side or agar plate, one *Rhizopus* sp with white color cottony colony and one *Mucor* sp. with white color colony. Further this isolates were identified morphologically by mounting them with lactophenol.

Selected isolates were screened for their alkaloid production. Among them a potential alkaloid producer *Aspergillus* sp. (black color) was selected for further studies (data not shown here). This isolate was designated as *Aspergillus* sp strain TAS1. Fungus showed all the four test positive except Wagner's test on the 10th day of incubation (Table - I). However, Wagner's test gave intense color after 20 day of incubation.

**Table 1 : Alkaloid production by *Aspergillus* sp. strain TAS1**

| No. | Test | Observation | Result | |
|---|---|---|---|---|
| | | | $10^{th}$ day | $20^{th}$ day |
| **1** | Mayer's Test | Cream colored precipitation | + | ++ |
| **2** | Dragendorff's Test | Orange colored precipitation | + | ++ |
| **3** | Wagner's Test | Red-brown colored precipitation | - | + |
| **4** | Hager's Test | yellow colored precipitation | + | +++ |

Characterization of alkaloid was done by thin layer chromatography. Under UV light five different spots were observed. However, on spraying with Ehrlich's reagent 3 bluish purple color spots were visualized. Rf values of spots were 0.4, 0.54 and 0.71. IR analysis revealed the bond present in the alkaloid structure. Peaks at 3418, 2922, 2852, 1720, 1635, 1458, 1381, 1276, 1123, 1066, 1056 $cm^{-1}$ indicates the presence of N-H, methylene, O-H, C-C, 1458,C=C, C-H, carboxylic acid, C-O, aliphatic amine overlapped respectively (Fig I). Thus, TLC and IR spectral analysis further confirm alkaloid production by the isolate.

Alkaloid production starts from $4^{th}$ day onwards and continue upto $20^{th}$ day of incubation. Maximum production was found on $20^{th}$ day of incubation (Fig II). Tryptophan has a central role in the biosynthesis of the ergot alkaloids. Addition of tryptophan increase alkaloid production by fungal isolate. Production was almost double fold on its addition. Induction of ergot alkaloid synthesis increased on tryptophan addition as supported by indirect evidence through increase in enzyme production responsible for alkaloid biosynthesis (Vining, 1970; Bu'Lock and Barr, 1968).

Growth of all the three isolates was inhibited by alkaloid as well as by standard antibiotics. Thus, alkaloid can be used to as antibacterial compound to control *E. coli*, *P. aeruginosa*, and *S. aureus*. Antioxidant assay by ABTS revealed that alkaloid comprised strong antioxidant activity.

  

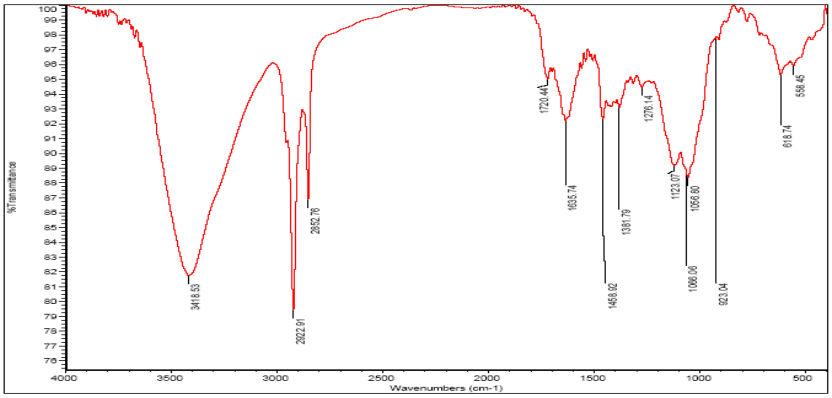


**Figure I – FTIR spectrum of alkaloid produced by *Aspergillus* sp. strainTAS1.**

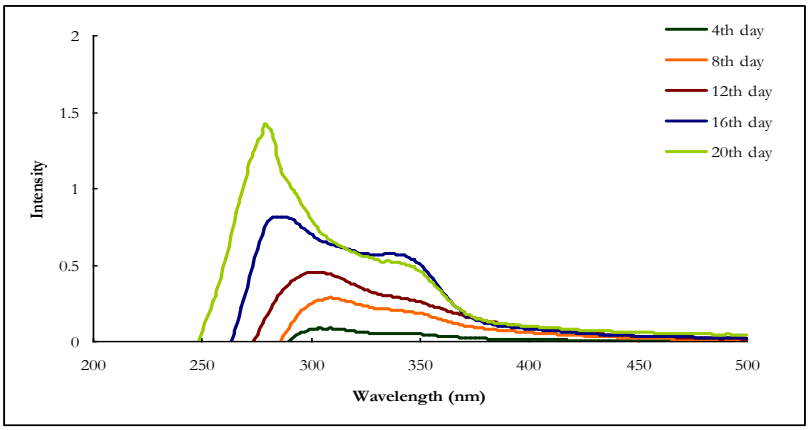


**Figure II – Temporal effect on alkaloid production by *Aspergillus* sp. strain TAS1**

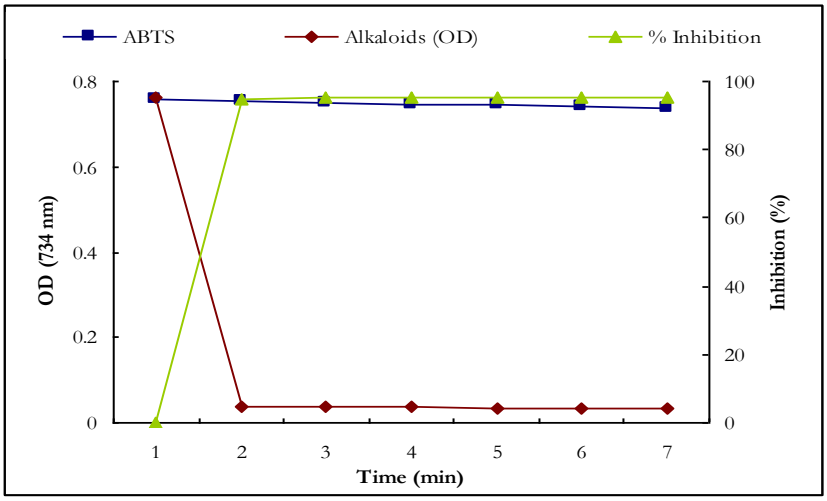


**Figure III – Antioxidant activity of alkaloid produced by *Aspergillus* sp strain TAS1**

  

After addition of sample into ABTS, after one min of incubation OD decreased almost to 0.039 which is further decreased to 0.033 after 7 min of incubation (Fig III). The percentage inhibition after in min was 94.8 and further increased to 95.5 % after 7 min of incubation.

## DISCUSSION

*Aspergillus* sp are well documented for production of ergot alkaloids. In present study, production of alkaloid by *Aspergillus* sp strain TAS1 was examined. Results demonstrate that high amount of alkaloid was produced by *Aspergillus* sp strain TAS1. Production was started from 4th day onwards and continue upto 20th day of incubation. Thus, it revealed that production was higher during idiophase. Delayed higher production indicates as they are the secondary metabolites, production was higher generally after 15 to 20th day of incubation. Isolate showed positive Dragendorff's test indicated presence of nitrogen containing compounds. Characterization by UV-Vis spectroscopy and IR analysis showed that it produced ergot alkaloid as it gave characteristic peak in UV range as shown in Figure II.

Tryptophan, in addition to serving as a biogenetic precursor for a portion of the ergoline ring system of the alkaloids, it may serve as an inducer of the enzyme required for alkaloid synthesis (Vining, 1970; Bu'Lock and Barr, 1968). Moreover, accumulate two to three fold tryptophan and twenty to twenty-five fold increase of tryptophan synthease activity during transition period between growth and the alkaloid production phase in fungal mycelium. Bu'Lock and Barr (1968) found that alkaloid production depends on rate of synthesis enzyme for alkaloid biosynthesis and the amount of tryptophan within the mycelium using tryptophan-supplemented cultures. Thus, results of increase in alkaloid production corroborate that addition of tryptophan increase its production.

The antioxidant status in human reflects the dynamic balance between the antioxidant defense and prooxidant conditions and this has been suggested as a useful tool in estimating the risk of oxidative damage (Tiwari, 2004). Ethyl acetate, methanol and aqueous extracts of the many medicinal mushroom like Ganoderma, Phellinus, Pleurotus sp have been effectively scavenge reactive oxygen generated during metabolism (Thekkuttuparambil and Janardhanan, 2007). *Aspergillus* sp strain TAS1 showed 95 % inhibition in ABTS assay indicates its potential as antioxidant agent. Thus, alkaloid can be used as useful tool to remediate oxidative damage. Fungal isolate *Aspergillus* sp. strain TAS1 isolated from soil and produced ergot type alkaloid as confirm by various biochemical test as well as by TLC and FTIR. Alkaloid inhibited growth of *E. coli, S. aureus* and *P. aeruginosa*. It possesses higher antioxidant activity. Thus, it can serve as potential sources of antioxidant and antibacterial compounds. However, intensive and extensive investigations are needed to exploit their valuable therapeutic use.

## ACKNOWLEDGMENT

Authors are thankful to Dr K C Patel Research and Development Centre, Changa for providing facility for FTIR analysis.

## REFERENCES

**Bu'Lock J.D. and Barr J.G. (1968)**. A regulation mechanism linking tryptophan uptake and synthesis with ergot alkaloid synthesis in Claviceps. *Lloydia. 31:342-354.*

**Camilo C., Lopes-Cardoso M.I., Whitmer S., Fits L.V.D., Pasquali G., Heijden e*t al.* (1998).** Effects of over-expression of strictosidine synthase and tryptophan decarboxylase on alkaloid production by cell cultures of *Catharanthus roseus. Planta. 205: 414-419.*

**Evans W.C. (2005).** Trease and Evans Pharmacognosy. 15th ed. New Delhi, India, A division of Reed Elsevier India Private Limited, Pp 20-22

**Flieger M., Wurst M. and Shelby R. (1997).** Ergot alkaloids - sources, structures, and analytical methods. *Folia Microbiol. 42:3–30.*

**Kozlovsky A.G. (1999).** Producers of ergot alkaloids out of *Claviceps* genus. Amsterdam. In: Kfen V, Cvak L , editors. Ergot: the genus *Claviceps*., The Netherlands: Harwood Academic Publishers, Pp. 479–499

**Kozlovsky A.G., Vinokurova N.G. and Adanin V.M. (2000).** Diketopiperazine alkaloids from the fungus *Penicillium piscarium* Westling. *Appl. Biochem. Microbiol. 3:271-275.*

  

**Radulovic N., Stankov-Jovanovic V., Stojanovic G., Smelcerovic A., Spiteller M. and Asakawa Y. (2007).** Screening of in vitro antimicrobial and antioxidant activity of nine Hypericum species from the Balkans. *Food. Chem. 103:15-21.*

**Re R., Pellegrini N., Proteggente A., Pannala A., Yang M. and Rice-Evans C. (1999).** Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Medicine. 26: 1231-1237.*

**Spilsbury J.F. and Wilkinson S. (1961).** The isolation of festuclavine and two new clavine alkaloids from *Aspergillus fumigatus*. *Fres. J. Chem. Soc. 5:2085-2091.*

**Thekkuttuparambil A.A. and Janardhanan K.K. (2007).** Indian medicinal mushrooms as a source of antioxidant and antitumor agents. *J. Clin. Biochem. Nutr. 40:157–162.*

**Tiwari A.K. (2004)**. Antioxidant: New generation therapeutic base for treatment of polygenic disorders. *Curr. Sci., 86:1092–1102.*

**Vining L.C. (1970).** Effect of tryptophan on alkaloid biosynthesis in cultures of a *Claviceps* species. *Can. J. Microbiol.* **16:473-480**

**Wagner H. (1993).** Pharmazeutische Biology. 5th ed. AUFI. 15 BN 3437-20 498-X. Gustav fisher Vwelag. Stuttgart.Germany, Pp 184.

**Wagner H.X.S. Bladt, Gain Z. and Suie E.M. (1996).** Plant drug analysis. Berlin,Germany. Springer Verlag, Pp360.

**Waldi D. (1965).** Spray Reagents for thin layer chromatography-A Laboratory handbook. New York (U.S.A): Academic press Inc.

  