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QUALITATIVE AND QUANTITATIVE ANALYSIS OF ERGOT ALKALOIDS PRODUCED BY *ASPERGILLUS NIGER* THROUGH SURFACE CULTURE FERMENTATION PROCESS

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Abstract

The present study deals with the qualitative and quantitative analysis of ergot alkaloids produced by *Aspergillus niger* using surface culture fermentation technique. Two different fermentation media (A and B) were prepared for the production of extracellular and intracellular extracts of *Aspergillus niger*. The fermentation media A and B were further modified as A1, A2, A3, A4, B1, B2, B3 and B4 respectively. After maintaining the pH of the media at 5.0 using 0.1N HCl and 0.1N NaOH, these flasks were incubated at 25°C for 10 days. After 10 days of incubation, the ergot alkaloids determination was done using spectrophotometry and thin layer chromatography (TLC) techniques. Maximum ergot alkaloids yield was obtained from extracellular and intracellular extracts of fermentation medium A4 (3.04± 0.1 mg/ml) and A2 (0.93±0.01 mg/ml) respectively. Likewise, maximum ergot alkaloids yield was achieved from extracellular (2.45±0.03 mg/ml) and intracellular (1.33±0.01 mg/ml) extracts of fermentation medium B3. For thin layer chromatographic studies, various mobile phases were screened and TLC plates of mobile phase G (chloroform 140: propanediol 55: water 15) revealed Van Urk reagent positive spots that produced remarkable pinkish purple color on silica gel plates. Maximum *Rf* value was measured from extracellular extracts of sample A3EE (*Rf*= 0.95) and intracellular extract of B3EE (*Rf* = 1.21) in mobile phase G which confirmed the presence of ergocryptine and ergotamine in the samples. After modification in the mobile phase G, maximum *Rf*value was obtained from extracellular extract of sample A3EE (*Rf*= 1.32) intracellular extract of B4ME (*Rf* =1.12) that also indicated the presence of ergocryptine and ergotamine in the extracts of *Aspergillus niger*.

Key words: Ergot alkaloids, Spectrophotometry, TLC (Thin layer chromatography), Extracellular and intracellular alkaloids.

Introduction

Ergot alkaloids are biologically active compounds produced by many species of fungi which are therapeutically, toxicologicallyand pharmacologically important secondary metabolites. Ergot alkaloids such as ergometrine, ergotamine, ergotoxine, lysergic acid and their derivatives are extensively used in the manufacturing of important drugs and for the treatment of various ailments. Previously, it was found that *Claviceps purpurea* (a member of family Clavicipiataceae) can produce a striking set of pharmacologically imperative ergot alkaloids. *Clavicepspurpurea* produces sclerotium after infecting rye seeds and these sclerotia are the reservoirs of secondary metabolites known as alkaloids (Kren, 1997; Devi & Prabakaran, 2014).

There are various biotechnological applications that can be used for the synthesis of ergot alkaloids using many species of fungi other than family Clavicipiataceae. Some members of the family trichocomaceae are capable of producing ergot alkaloids such as *Aspergillus fumigatus* and *Penicillium commune.* The profiles of ergot alkaloids achieved by these species make them potential candidates for the production of clavine-type ergot alkaloids (Flieger *et al.,* 1997; Peter & Shu-Ming, 2013; Shahid *et al.,* 2017). Many species of genus *Aspergillus* can infect various plants and are able to produces toxic metabolites in plants which can influence nervous and reproductive system of the animals that feed on these contaminated plants. Previously, it was observed that, ergotamine, ergoclavine, festuclavine and fumigaclavine are produced by *Aspergillusfumigatus*and*Aspergillus niger* which can be used to cure hypertension, half headed aches and to reduce post partum bleeding (Coyle *et al.,* 2007). Researchers are also working on conidia synthesizing genes in strains of *Aspergillus fumigatus* so that it would be able to produce conidia during its reproduction. Therefore, a bristle A gene (brlA) has been identified as the necessary gene for the conidial development in the *Aspergillus fumigatus*. The profiles of ergot alkaloids have been identified by various investigative processes such as colorimetry, spectrophotometry, thin layer chromatography and HPLC techniques. Many researchers have developed some simple techniques for the identification of ergovaline in leaves of tall fescue grass (*Festuca arundinacea*) (Adriana & Godoy (2001). The Thin layer chromatography method has been used for the identification and quantification of caffeine, ergotamine and metamizol in different standard drugs. These compounds can be synthesized in laboratory using various fermentation techniques such as solid state, submerged and surface culture processes (Shahid *et al.,* 2016. The components of ergot alkaloids can be alienated on silica gels plates using High Performance

Thin Layer Chromatography (HPTLC) using the mixture of some organic solvents such as ethylacetate: methanol: ammonia in 90:15:1 ratio (Aranda & Morlock, 2007). Caffeine, metamizol, ergotamice and amyloclavine can be observed using UV lamp ane fluoresce dye at 274 nm and 340 nm respectively. These days, mass spectrometry and positive electrospray ionization process are in use for the identification of caffeine and ergotamine type of secondary metabolites. The synthesis of these compounds in laboratories can be cost effective and alternative source for the detection of ergot alkaloid compounds (Shahid *et al.*, 2016). In the present study, quantitative and qualitative methods such as spectrophotometry and TLC analyses were used for the detection and separation of ergot alkaloid compounds produced by *Aspergillus niger* grown after biologically synthesizing these compounds using surface culture fermentation technique.

Materials and Methods

Procurement and maintenance of fungal strain: The strain of *Aspergillus niger* was collected from Institute of Industrial Biotechnology, GC University, Lahore and grown on PDA medium slants. PDA slants were prepared by dissolving 4g Potato Dextrose Agar (PDA) in 100 ml of distilled water and after inoculation with *Aspergillus niger* strain, these slants were incubated at 25°C for 5 days. The fully grown slants were kept at 4°C for supplementary analysis.

Preparation of inoculum: The inoculum was prepared by pouring 5 ml of distilled water in a fully grown slant and vortex for 2-3 times for proper mixing of the spores in the distilled water. The colonies of *Aspergillus niger* were scrapped by spatula in the test tube. One ml of spore suspension contains 10^{6-7} spores.

Preparation of fermentation broth for ergot alkaloids production: Two types of fermentation media were prepared to obtain the yield of ergot alkaloids using *Aspergillus niger* under surface culture fermentation conditions.

Fermentation medium A: It was prepared by adding 10 g sucrose, 20 g beef extract, 1 g tryptophan, 1 g asparagine, 1 g KH_2PO_4, 0.2 g $MgSO_4$, 0.2 g $FeSO_4$ and 0.2 g $ZnSO_4$ in a 250 ml Erlenmeyer flask containing 100 ml of distilled water. The fermentation medium A was further modified into A1, A2, A3 and A4. The recipe of the modified fermentation media was as follows:

i. A1: 15 g sucrose, 10 g beef extract, 2 g tryptophan, 1 g asparagine, 1 g KH_2PO_4, 0.5 g $MgSO_4$, 0.5 g $FeSO_4$ and 0.5 g $ZnSO_4$ in 100 ml of distilled water.
ii. A2: 20 g sucrose, 20 g malt extract, 1 g peptones, 2 g KH_2PO_4, 0.2 g $MgSO_4$, 0.2 g $FeSO_4$ and 0.2 g $ZnSO_4$ in 100 ml of distilled water.
iii. A3: 25 g sucrose, 10 yeast extract, 2 g tryptophan, 1 g asparagine, 0.5 g $MgSO_4$, 0.5 g $FeSO_4$ and 0.5 g $ZnSO_4$ in 100 ml of distilled water.
iv. A4: 30 g sucrose, 30 g beef extract, 4 g tryptophan, 2 g asparagine, 1.5 g KH_2PO_4, 0.5 g $MgSO_4$, 0.5 g $FeSO_4$ and 0.5 g $ZnSO_4$ in 100 ml of distilled water.

1. Fermentation medium B: It was prepared by adding 10 g mannitol, 20 g yeast extract, 1 g KH_2PO_4, 0.2 g $MgSO_4$, 0.2 g $FeSO_4$ and 0.2 g $ZnSO_4$ in a 250 ml Erlenmeyer flask containing 100 ml distilled water. This fermentation medium was also modified into self constructed fermentation media such as B1, B2, B3 and B4. The recipe which was used is as under:

i. B1: 10 sucrose, 5 g yeast extract, 1 g KH_2PO_4, 1 g $MgSO_4$, 1 g $FeSO_4$ and 1g $ZnSO_4$ in 100 ml distilled water.
ii. B2: 10 rise husk, 5 g beef extract, 1 g KH_2PO_4, 1 g Ammonium chloride, 1 g $MgSO_4$, 1 g $FeSO_4$ and 1g $ZnSO_4$ in 100 ml distilled water.
iii. B3: 30 g glucose, 20 g malt extract, 2 g K_2HPO_4, 2 g asparagine, 1 g $FeSO_4$ and 1g $ZnSO_4$ in 100 ml distilled water.
iv. B4: 30 sucrose, 20 g yeast extract, 1 g KH_2PO_4, 1g tryptophan, 1 g $MgSO_4$, 1 g $FeSO_4$ and 1g $ZnSO_4$ in 100 ml distilled water.

The pH of above mentioned media was adjusted at 5.0 using 0.1N HCl and 0.1N NaOH. The flasks were autoclaved at 121°C, 15lb pressure for 20 min. After sterilization, fermentation medium A and B were inoculated with 5 ml of spore suspension of *Aspergillus niger*. All the flasks after inoculation were incubated at 25°C for 10 days.

Determination of ergot alkaloids: The fermented broth (extracellular) and the mycelia (intracellular) were separated after fermentation process in separate glass bottles.

Extracellular alkaloid extracts: Fermented broth was subjected to centrifugation process at 10,000 rpm at 4°C for 10 min. After centrifugation, supernatant was collected in separate glass bottle and subjected to chloroform extraction process to get the pure extract of ergot alkaloids. These extracellular extracts were stored at 4°C for further investigation of ergot alkaloids.

Intracellular alkaloid extracts: The mycelia that were alienated from fermentation broths of *Aspergillus niger* were initially weighed and located in oven at 40°C for 24 h for drying purpose. After 24 h dried mycelium were weighed again. These dried mycelia were dipped in 20 ml of methanol and placed at room temperature for 2-3 hours. These dipped mycelia were subjected to cell lysis using sonication process for 6 min at 200 rpm/min in an ultrasonic generator. A homogenizer was used for 10 min to homogenize the contents of mycelia so that all contents of ergot alkaloids may be released from the mycelia of *Aspergillus niger* in to the extract (Linde, 2005).

Determination of ergot alkaloids

Quantitative and qualitative analysis of ergot alkaloids

Spectrophotometry: 1 ml of each extracellular and intracellular filtrate was mixed with 2 ml of Van Urk's Reagent (Van Urk, 1929; Smith, 1930) in test tubes and

total volume was raised upto 3 ml. The reaction mixture was incubated at 37°C for 30 min in a water bath. After 30 min a change in the color of the reaction mixture was observed. The optical density of all the reaction mixtures was measured at 590 nm using spectrophotometer. The presence of ergot alkaloids produced was observed by noticing purple brown color in the reaction mixture and compared with reference compounds of ergot alkaloids i.e. dihydroergotamine methane sulfonate, ergotamine and bromocryptinemesylate salts.

Qualitative analysis of ergot alkaloids

TLC Analysis: Extracts of ergot alkaloids produced by *Aspergillus niger* were subjected to chloroform extraction process. 10 ml of chloroform was added in the 5 ml of extract of *Aspergillus niger* in a separating funnel. The mixture was shaken three times to make two layers of extract. The upper layer was discarded and lower layer was again mixed with chloroform. This process was repeated for three times. After this process the lower layer of extract was this again purified using rotary evaporator. The residues were then analyzed for the ergot alkaloids presence by spectrophotometery and thin-layer chromatography processes. For TLC analyses following two steps were performed.

STEP-I (Screening of mobile phases for TLC analysis): For this purpose, a range of mobile phases were prepared and screened for the presence of ergot alkaloids in extracellular and intracellular filtrates synthesized by *Aspergillus niger*. Different mobile phases of organic solvents were prepared using various recipes. The proportions of the mobile phases used are given in the Table 1.

STEP-II (Final mobile phase for TLC analysis): For the finalization of screened mobile phase for TLC analysis, the mobile phase "G" was chosen from the screened TLC mobile phases. The mobile phase "G" was modified having a mixture of various organic solvents to achieve the maximum separation of ergot alkaloids. The composition and proportion of "G" mobile phase is shown in the Table 2.

Table 1. Screening of mobile phases for TLC analysis.

Mobile phase	Composition of solvents
A	Chloroform: Methanol: Ammonia Solution (70:30:0.5)
B	Chloroform: Acetone : Acetic acid (80: 20: 5)
C	Chloroform : Ethanol (90: 10)
D	Chloroform: Acetone: Orthophosphoric acid (90: 10: 5)
E	Methanol: Ethyl acetate (40: 60)
F	Chloroform : Ethanol (80:20)
G	Chloroform : Propanediol : Water (140:55:15)

Table 2. Selected mobile phase for TLC analysis.

Mobile phase	Composition of solvents
G1	Chloroform : Propanediol: Water (120:30:15)
G2	Chloroform : Propanediol: Water (120:30:20)
G3	Chloroform : Propanediol :Water (100:40: 30)
G4	Chloroform : Propanediol :Water (140:40: 20)
G5	Chloroform : Propanediol :Water (90:60: 50)

These TLC plates were placed in a horizontal TLC chamber after applying a 3µl spot of the standard salt solution "dihydroergotamine methane sulfonate salt (DMS) and BromocryptineMesylate (BCM). After applying the spot of standard salt, the 3 µl spots of the extracellular and intracellular extracts were also applied separately on silica gel plates. The spotted silica gel plates were placed in chromatographic chambers containing organic solvents in them. After chromatography, TLC plates were allowed to dry under room temperature. After their drying, Van Urk reagent was sprayed on the spotted part of TLC plates. These TLC plates were kept in UV lamp at 254 nm to detect the presence of ergot alkaloids and within a few minutes a pinkish purple color was observed on these plates. R*f* values of all the spots were calculated using the formula mentioned below. Two silica gel plates were prepared for organic mobile phase (Polak & Rompala (2007). R*f* values of supernatant (extracellular) and mycelial (intracellular) filtrates/ extracts were noted using the following formula:

$$Rf = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent}}$$

Results and Discussion

Quantitative and qualitative determination of ergot alkaloids

Spectrophotometry: *Aspergillus niger* was obtained from the Institute of Industrial Biotechnology, GC University, Lahore. *Aspergillus niger* was observed as a best producer of ergot alkaloids in various both fermentation medium A and B. But ergot alkaloids concentration was 2 folds higher in fermentation medium A4 as described in the Table 3. Mycelial growth was also determine in both of the fermentation media and maximum growth of mycelia was obtained in fermentation medium B3 (10.53 g/100 ml) and lowest amount was observed in sample no. A1 (2.6g/100ml) as presented in Fig. 1.

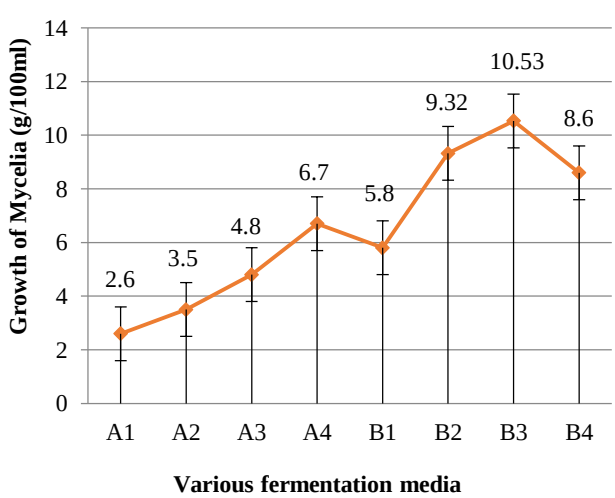

Fig. 1. Growth of mycelia in various fermentation media.

Table 3. Yield of ergot alkaloids produced by *Aspergillus niger*.

Fermentation medium A			Fermentation medium B		
Sample No.	**Extracellular ergot alkaloids (mg/ml)**	**Intracellular ergot alkaloids (mg/ml)**	**Sample no.**	**Extracellular ergot alkaloids (mg/ml)**	**Intracellular ergot alkaloids (mg/ml**
A1	1.29 ± 0.01	0.51 ± 0.01	B1	2.20 ± 0.01	1.09 ± 0.01
A2	1.32 ± 0.02	0.93 ± 0.01	B2	1.91 ± 0.01	0.94 ± 0.02
A3	2.16 ± 0.1	0.56 ± 0.1	B3	2.45 ± 0.03*	1.33 ± 0.01*
A4	3.04 ± 0.1*	0.32 ± 0.01	B4	2.04 ± 0.02	0.96 ± 0.01

"±" Indicates the standard deviation among three replicates

Maximum ergot alkaloids production was achieved from intracellular (mycelial) extract of sample No. B3 of *Aspergillus niger* that was grown in fermentation medium B (1.33 mg/ml). Lowest alkaloids production was also observed in intracellular extract of fermentation medium A4 (0.32 mg/ml) as described in Table 3

Table 4. *Rf* values of filtrates of *Aspergillus niger* in screening phase.

Filtrate No.	Mobile Phase and *Rf* Value							Expected ergot alkaloids
	A	**B**	**C**	**D**	**E**	**F**	**G**	
	Fermentation media A							
A1EE,	0.13	-	-	-	0.22	-	0.54	Ergocryptine
A1ME	0.12	-	-	-	0.01	-	0.42	Agroclavine Ergotamina
A2EE,	-	-	-	-	-	-	-	-
A2ME	-	-	-	-	-	-	-	-
A3EE,	0.15	0.04	0.07	0.07	0.06	0.05	**0.95**	Ergocryptine
A3ME	0.21	0.12	0.05	0.06	0.05	0.06	**0.70**	Ergotamine
A4EE	0.31	-	0.25	0.21	-	-	0.52	Ergocryptine Agroclavine
A4ME	0.29	-	0.14	0.12	-	-	0.31	Ergotamine
	Fermentation media B							
B1EE	0.32	0.15	0.34	-	0.12	0.47	0.49	Ergocryptine
B1ME	0.30	0.19	0.39	0.32	0.09	0.38	0.56	Ergotamine
B2EE	0.29	0.24	0.21	0.11	0.15	0.57	0.78	Ergocryptine
B2ME	0.34	0.38	0.32	0.32	0.16	0.61	0.51	Ergocryptine
B3EE	0.45	0.37	0.56	0.46	0.98	0.99	**1.21**	Ergocryptine
B3ME	0.52	0.41	0.51	0.78	0.65	0.78	**0.99**	Ergotamine Emyloclavine
B4EE	0.14	0.44	0.61	0.71	0.77	0.98	0.81	Ergocryptine
B4ME	0.23	0.52	0.34	0.69	0.67	0.56	0.87	Emyloclavine Ergocryptine

Where A & B: Fermentation medium; EE: Extracellular extract; ME: mycelial extract (intracellular extract)

Thin Layer Chromatography (TLC)

Step I (Screening of various mobile phases for ergot alkaloids: Thin layer chromatography (TLC) was done on silica gel plates (60G, F_{254}) and maximum R*f* value was obtained from extracellular extract of fermentation medium A (A3EE:0.75) and mycelial extract of fermentation medium A (A3ME:0.70) in mobile phase G (chloroform: propanediole: water (140:55:15). Lowest *Rf*value was obtained from extracellular extract of fermentation medium A (A3EE:0.04) and mycelial extract of fermentation medium A (A1ME:0.01) in mobile phases B and E respectively. The characteristic pinkish purple and bluish purple colored spots were achieved on silica gel plates that indicated the presence of ergocryptine and ergotamine respectively in the extracts of self constructed fermentation media A produced by *Aspergillus niger* as described in Table 4.

The *Rf*values of extracts of fermentation media B in which mobile phases F and G were considered as the best to obtain the maximum value (Table 4). Highest *Rf*value was obtained from the extracellular (B3EE:1.21) and mycelial (B3ME:0.99) extract of fermentation medium B3. Least *Rf*value was measured from the extracellular (B2EE:0.11) and mycelial (B1ME:0.09) extracts of fermentation media B2 and B1 respectively as shown in Fig. 2.

STEP-II (TLC of Ergot alkaloids of selected extracts in mobile phase G): After step 1 of TLC, mobile phase G was selected as the final mobile phase for the running of ergot alkaloids. The G mobile phase was modified into various phases such as G1, G2, G3, G4 and G5 for the complete separation of ergot alkaloid present in extracellular and intracellular filtrate of *Aspergillus niger*.

In this step, maximum *Rf*value was measured from extracellular and intracellular (mycelial) filtrates of A3EE and A3ME with *Rf* of 0.99 and 0.98, respectively in mobile phase G4 (Chloroform 140: Propanediol 40: Water 20). In extracts of fermentation medium B3, extracellular (B3EE:0.87) and mycelial (B3ME:0.89) also gave the maximum separation in mobile phase G. In another selected extract of fermentation medium B4, maximum separation of compounds was achieved in mobile phase G5 with a value of 0.98 in extracellular and 1.12 in mycelial extracts respectively as shown in Table 5 (Fig. 3).

Table 5. *Rf*values of extracellular and mycelial extracts of *Aspergillus niger*.

Extract No.	Mobile phase and *Rf*value					Expected ergot alkaloid compounds
	G1	G2	G3	G4	G5	
A3EE	0.76	0.95	0.67	**1.32**	0.91	Ergocryptine
A3ME	0.54	0.71	0.62	0.98	0.93	Ergotamine
B3EE	0.42	0.51	0.49	0.87	0.79	Ergocryptine
B3ME	0.40	0.48	0.42	0.89	0.67	Ergotamine
B4EE	0.88	0.86	0.67	0.77	0.98	Ergocryptine
B4ME	0.78	0.83	0.60	0.79	**1.12**	Ergotamine

Where A & B: Fermentation medium; EE: Extracellular extract; ME: mycelial extract (intracellularextract)

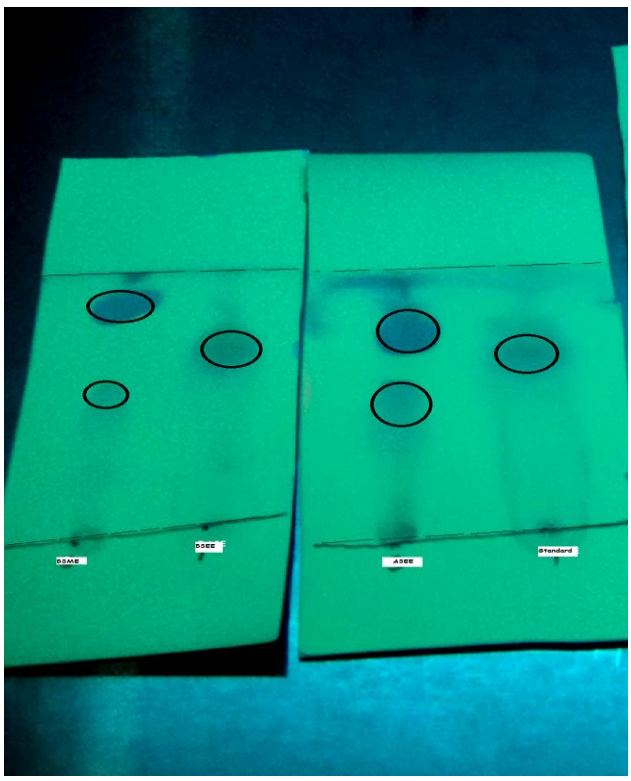

Fig. 2. Movement of ergot alkaloids in mobile phase G of fermentation medium B, Where S is standard salt of Dihydroergotamine Methane Sulfonate Salt (DMS).

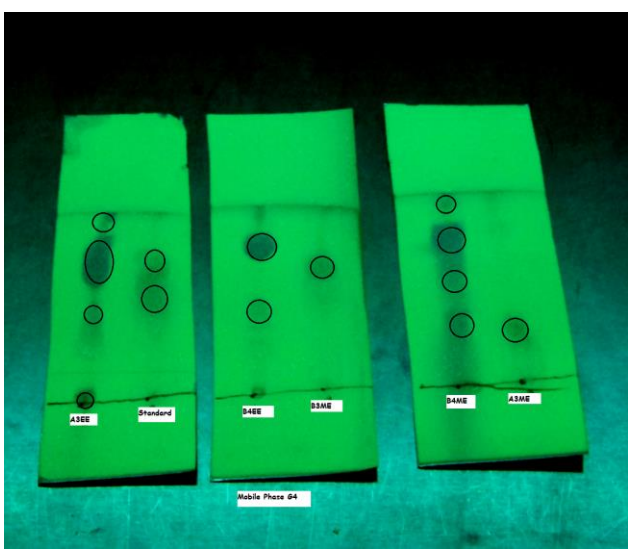

Fig. 3. Maximum *Rf* value in G5 mobile phase for the extract A3EE and A3ME. Where S represents the standard salt of ergot alkaloids (Bromocryptinemesylate).

Discussion

Secondary metabolites are produced by many microorganisms especially fungi are known as natural microbial products. These low molecular weight secondary metabolites may have significant physiological activities. Ergotamine, ergocryptine, ergosonine and LSD (lysergic acid alkaloids) are important fungal secondary metabolites. All secondary metabolites are produced through common biosynthetic pathways in combination with their morphological development (Keller, 2005). Many fungal species belong to two diverse families of Ascomycetes such as Clavicipitaceae and Trichocomaceae which can produce various ergot alkaloids which are being used in pharmacology and agriculture. In past, the fungal ergot alkaloids in the form of sclerotia of *Claviceps purpurea* were used by midwives to induce labour pains and to reduce post-partum bleeding. Now a days, lysergic acid alkaloids and ergotamine derived drugs are used to cure Alzhemier, dementia, type 2 diabetes and hyperprolactinemia diseases (Baskys & Hou, 2007; Morren & Galvez-Jimenez, 2010; Perez-Lloret & Rascol, 2010; Kerr *et al.*, 2010).

The present study deals with the thin layer chromatography of ergot alkaloids produced from *Aspergillus niger* under surface culture fermentation conditions. Various mobile phases were prepared for the separation of ergot alkaloids contents of extracellular and intracellular extracts of *Aspergillus niger*. In the first step of TLC, screening of various mobile phases was done and maxium *Rf* value was obtained in G (Chloroform 140: Propanediol 55: Water 15) mobile phase (*Rf*: 0.99) in B3ME extract of *Aspergillus niger* as described in Table 4. Similar solvent system was used by Agurell (1965) for separation of ergot alkaloids (Lysergic acid). This solvent system had the following composition: (Methanol 4: Chloroform 3: Acetic acid 3 v/v). It was concluded that using this solvent system, both the isolated sample and authentic specimen of lysergic acid produced *Rf* value of 0.28 when examined on thin layer chromatography plates. Prosek *et al*., (1976) also used various conditions for the in situ analysis of ergot alkaloids on TLC by fluorescence scanning method. They measured excitation and emission spectra of ergot alkaloids and recorded their peak areas. It was concluded that fluorescence scanning method was the most suitable method to quantify ergot alkaloids after separation by TLC because this methods needed only nanogram quantities of samples containing ergot alkaloids. Polak & Rompala (2007) used similar solvent system and concluded that adding up of acids to the mobile phase can somewhat reduced the binding of alkaloid molecules by the silica gel surface and can slightly increase the *Rf* values of the alkaloids. The maximum *Rf* values were obtained with methanol: ethyl methyl ketone mobile phases. The eluent

strength of mobile phases containing acid depended not only on the type of acid but also on the other components of the mobile phase.

In the present study, after the screening of various mobile phases, G (Chloroform 140: Propanediol 55: Water 15) mobile phase was selected as the final mobile phase for the separation of ergot alkaloid contents. The mobile phase G was further modified in G1, G2, G3, G4 and G5 mobile phases. The maximum *Rf* value was measured in mobile phase G5 as *Rf* of 1.12 as shown in Table 5 which depicted that maximum separation of ergot alkaloids was achieved in B3ME extract of *Aspergillus niger*. The TLC analysis of the samples revealed three Ehrlich's reagent positive spots on TLC plates showing pinkish purple color of ergot alkaloids. This color revealed that in the samples of *Aspergillus niger* ergocryptine and ergotamine contents were present. Our results were in concordance with the results of Moussa (2003) who worked on the ergot alkaloids produced by different various species of *Penicillium* and achieved an *Rf*value of 0.70 under UV lamp showing a pinkish purple color on TLC plates. His work also concluded that in various extracts of ergot alkaloids, ergocryptine was the major part of the contents of mycelial and supernatant extracts. He also identified ergot alkaloids as agroclavine and elymooclavie when *Rf*value was measured as 0.40 and 0.28 respectively. It is concluded that in this study, ergotamine, ergocryptine, agroclavine and emyloclavine are present in the extracellular and intracellular extracts of *Aspergillus niger*. Adriana & Godoy (2001) also worked on a relatively low-cost TLC technique to separate the contents of ergot alkaloids in the extracts of leaves of *Festuca arundinacea*. They used the mobile phase of choloroform, acetone and acetic acid in ratio of 90:10:5 and chloroform and ethanol in ration 9:1 ratio for TLC investigation. The TLC plates were experimented under UV light after spraying *p*-dimethylaminobenzyldehyde and sulfuric acid on the plates. After a few minutes of reaction, they observed a pinkish purple color which indicated the presence of ergoline alkaloid in the leaves of the plant. They also obtained an *Rf* values that was compared with the standard ergotamine alkaloid. The *Rf*value for ergoline was obtained in the range of 0.40-0.60. They concluded that TLC method was a very cost effective method to categorize ergot alkaloids at initial stage of their analysis. So this TLC method can also be used as a pilot method for further identifications through HPLC method.

Conclusion

Ergot alkaloids are pharmacologically important because of the toxicity of their contents. They are useful in the mitigation of various diseases such as migraine, Alzheimer's disease and can help to stop prolactin. They can also act as serotonin antagonists, can terminate early pregnancy and inhibit mammary tumors. Ergot alkaloids are produced from various species earlier and in this study, *Aspergillus niger* has been found as a potential candidate for the production of ergot alkaloids. Ergocryptine, ergotamine, agroclavine and emyloclavine were also identified through TLC in this study, which are considered as pharmacologically significant alkaloids.

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