WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 1360-1369. Research Article ISSN 2277– 7105

EVALUATING THE ANTI-SHIGELLOSIS ACTIVITY OF FEW EDIBLE MUSHROOMS AGAINST MULTIDRUG RESISTANT SHIGELLA SP.

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Article Received on
27 April 2015,

Revised on 21 May 2015,
Accepted on 13 June 2015

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ABSTRACT

Mushrooms are of immense significance in the field of research for many years, because of its high nutritional and pharmaceutical importance. Many wild and edible mushrooms have showed varied medicinal importance be it antineoplastic, antiatherosclerotic, antiviral and antimicrobial activities. In the present study we have showed a comparative analysis of antibacterial activities of alcoholic and aqueous extracts of five edible mushrooms of genus *Pleurotus* and *Calocybe* specifically *P.ostreatus*, *P.florida*, *P.eous*, *P.sajor-caju* and *C.indica* against *Shigella* spp., as very few research studies are available against Shigellosis, which is a prevalent dysenteric disease in developing countries especially in children. In this study we have also studied antibacterial activity against one multidrug resistant *Shigella* sp. A detailed comparative analysis of the phytochemical constituents of all the mushroom extracts was done and the antimicrobial activity was correlated with the phytochemical constituents of the mushrooms. Results showed that only the ethanol extracts of all the mushrooms showed potent activity against the multidrug resistant strain showing MIC in the range of 0.94mg/mL- 15mg/mL. The enhanced antibacterial activity of the ethanol extract in comparison to the hot water extract was probably due to the significant difference (Two way ANOVA, P value < 0.0001) of the secondary metabolite -phenol content of the extracts. The results also indicated possible higher phenol content is responsible for maximum antibacterial activity of oyster mushrooms *P.ostreatus*.

KEYWORDS: Mushrooms, antibacterial activity, Shigellosis, phenol compounds.

INTRODUCTION

According to the definition of Chang and Miles, the term "mushroom" means - "a macrofungus with a distinctive fruiting body, which can either be hypogeous or epigeous, large enough to be seen with naked eye and picked up by hand". [1] It is a nutritious vegetable source and an excellent alternative to meat and eggs. Cultivation of mushroom is a subject of more than 100 years and the production of specifically oyster mushroom has increased sharply during the last decade.[2] The health enhancing activity of mushrooms are due to the presence of many bioactive substance such as phenols, terpenoids, flavonoids etc. Some bioactive substances have been referred as "biological response modifiers" as they enhance and modulate our body's reaction to an infection.[3, 4] Surprisingly near about 1,40,000 mushrooms exist on earth and only 10% out of that are known to us.[3] Among all the edible group of mushrooms oyster mushrooms are of immense value. It has worldwide importance and many species are grown in large or small scale in many countries.[2, 11] This group of mushrooms contains many bioactive components such as phenols, terpenoids, tannins responsible for its antibacterial activities and flavonoids for its excellent property of biological response modifiers as it modifies our body's reaction to many allergic reactions.[4] A detailed study reported about the presence of various phenol compounds such as p-hydroxybenzoic acid, protocatechuic acid, gallic acid, vanillic acid, syringic acid, cinnamic acid, p-coumaric acid, ferilic acid, quercetin, rutin, chrysin in edible oyster mushrooms namely *P.ostreatus*, *P.sajorcaju*, *P.djamor*.[5]

As increasing resistance to commercially available antibiotics are becoming a serious problem. The antibiotics that are working today may not prove beneficial tomorrow. Very few studies are available regarding the antibacterial properties of edible mushrooms against *Shigella* sp. As Shigellosis is a prevalent enteric disease in all developing countries among children and majority (85%) of the *Shigella* sp. are isolated from fresh stools or rectal swab samples showed multidrug resistance property and higher MIC values against commonly used antimicrobials like ampicillin, tetracycline, nalidixic acid and co-trimoxazole. Resistance to fluoroquinolones emerged alarmingly among *S.flexneri* subtypes (2a and 3a) in 2004 which restricted the use of fluoroquinolones for treatment.[6] It is a necessity to curb down the growing antimicrobial resistance among *Shigella* spp. as it is endemic disease in most developing countries.

In the present study the antibacterial potency of aqueous and alcoholic extracts of five edible mushrooms were screened. Phytochemical constituents screening of the extracts were also done to figure out the presence and estimation of natural antimicrobial components.

MATERIALS AND METHODS

Collection of Mushroom samples and microorganisms

Edible mushroom samples were collected from local mushroom farm of Narendrapur Ramakrishna Mission, South 24 Parganas, West Bengal, India. Fresh edible mushroom samples such as *Pleurotus ostreatus*, *Pleurotus eous*, *Pleurotus florida*, *Pleurotus sajor-caju*, *Calocybe indica* were obtained from the cultivation unit.

WHO reference strains of *Shigella* spp. namely *S.flexneri* type 4a, *S.boydii*, *S.sonnei*, were received from Peerless Hospital and B. K. Roy research centre, Kolkata, India. Multidrug resistant strain of *S.flexneri* type 2a (COT^R, S^R, NA^R, AMC^R) was collected from National Institute of Cholera and Enteric Diseases.

Extract Preparation

Alcohol Extract Preparation

Fresh mushroom samples were shade dried and powdered. The powdered material was extracted with 60% ethanol for about 72 hours in dark at room temperature. The extracts were then filtered with Whatman filter paper number 1, and then sterilized with 0.22 micron syringe membrane filter. The extracts were lyophilized and the crude content was measured and was freeze dried.

Aqueous Extract Preparation

Hot aqueous extracts were prepared by boiling coarse mushroom powder in distilled water for 3 hours and then cooled to room temperature. The extracts were then filtered with Whatman filter paper number 1, and then sterilized with 0.22 micron syringe membrane filter. The extracts were lyophilized and the crude content was measured and was freeze dried.

Antibacterial Assays

Zone of Inhibition assays

0.5 MacFarland opacity inoculums of the bacterial strains were made and a uniform lawn culture is done with sterile cotton swab stick on Antibiotic Susceptibility agar. The plates

were allowed to soak for 15 minutes. Discs made of Whatmann filter paper of 6mm diameter were prepared and were made sterile by autoclaving. The discs were placed upon bacterial lawn culture and were impregnated with various extracts. Then the plates were again soaked in refrigerator for about 15 minutes. Then the plates were incubated at 37 ºC for about 16-18 hours. The zones were observed the next day and the diameters were recorded. Every time a solvent control is always kept.[2]

Minimum Inhibitory Concentration Assays

0.5 MacFarland opacity suspension of the bacterial strains was prepared for the assay. The MIC assay was performed in microtitre plate. Previously 100 µl of broth was added in all the wells and 100 µl of extract was given in the first well. Then serial dilution was made. The sets were done in triplicate. After the serial dilution of the extracts, 10 µl of culture was inoculated in each well. It was mixed properly by shaking and was incubated at 37 ºC for 16-18 hours. The absorbance was measured at 0 hour and at 18th hour and the inhibition using 96 well plate reader and growth of the bacterium is determined.[10]

Phytochemical Screening Assays

All the phytochemical screening assays were evaluated using standard protocols.[2, 7, 8, 9]

Test for Flavonoid

1mL of stock solution of the extracts was taken in test tubes. Few drops of dilute sodium hydroxide solution were added followed by few drops of dilute hydrochloric acid. An intense yellow color develops and becomes colorless on addition of acids indicated the presence of flavonoids.

Test for Terpenoids

0.5mL of extracts was taken in test tubes and added 2mL of chloroform. Concentrated sulfuric acid was added very carefully. A reddish brown layer develops at the interface which indicates the presence of terpenoids.

Test for Steroids

0.2mL of each extracts were taken in test tubes and 2mL of acetic acid was added. The solution was cooled in ice and concentrated sulfuric acid was added carefully. Development of violet to blue colored ring indicated the presence of steroids.

Test for Tannins

The extracts were diluted with distilled water in a test tube. Few drops of 10% ferric chloride were added development of brownish green or blue-black coloration indicated the presence of tannins.

Test for Saponins

The ethanol extract was diluted in distilled water and was vigorously shaken. The appearance of stable persistent froth indicated the presence of saponins.

Estimation of Total Phenol Content (TPC) by modified Folin Ciocalteau method

Total phenolic content (TPC) was determined by modified Folin Ciocalteau reagent method. 10% of Folin Ciocalteau was added to 200µL of crude extracts. Sample kept in dark for about 5 minutes. Then added 5% sodium carbonate solution and mixed properly. The sample was kept in dark incubation for 2 hours and absorbance was measured at 700nm. The phenol content was estimated from the standard curve equation using pyrocatechol as a standard.

Estimation of Total Flavonoid Content (TFC)

Total Flavonoid Content was measured using aluminium chloride assay. To 2mL of distilled water added 0.5 mL of extracts and subsequently added 150 µL of 5% sodium nitrate solution. After 6 minutes added 150 µL of 10% aluminium chloride solution and again allowed to stand for 6 minutes. 2mL of 4% sodium hydroxide solution was added and allowed to stand for another 15 minutes. The absorbance was taken at 450nm. The standard curve was prepared using pyrocatechol as a standard and the concentration was measured using the standard curve equation.

RESULTS: The results are given in Table 1-3, Fig. 1-7.

Table 1: Phytochemical screening assay of the ethanol extracts of the five edible mushrooms.

Mushroom Extracts	Phenol	Flavonoid	Terpenoid	Steroid	Saponins	Tannins
P.ostreatus	+	+	+	+	+	-
P.eous	+	+	+	+	+	-
P.florida	+	+	+	+	+	-
P.sajor-caju	+	+	+	+	+	-
C.indica	+	+	+	+	+	-

+: Present; -: Absent

Table 2: Phytochemical screening assay of the hot water extracts of the five edible mushrooms

Mushroom Extracts	Phenol	Flavonoid	Terpenoid	Steroid	Saponins	Tannins
P.ostreatus	+	+	+	+	+	-
P.eous	+	+	+	+	-	-
P.florida	+	+	+	+	+	-
P.sajor- caju	+	+	+	+	+	-
C.indica	+	+	+	+	+	-

+: Present; -: Absent

Table 3: Zone of Inhibition assay against the ethanol extract of the mushrooms (in mm)

Mushroom Extracts	*S.boydii* (in mm)	*S.sonnei* (in mm)	*S.flexneri* type 4a (in mm)	*S.dysenteriae* type 2a MDR (in mm)
P.ostreatus	10	11	12	12
P.eous	10	10	11	12
P.florida	12	11	12	12
P.sajor-caju	12	12	11	11
C.indica	13	11	11	12

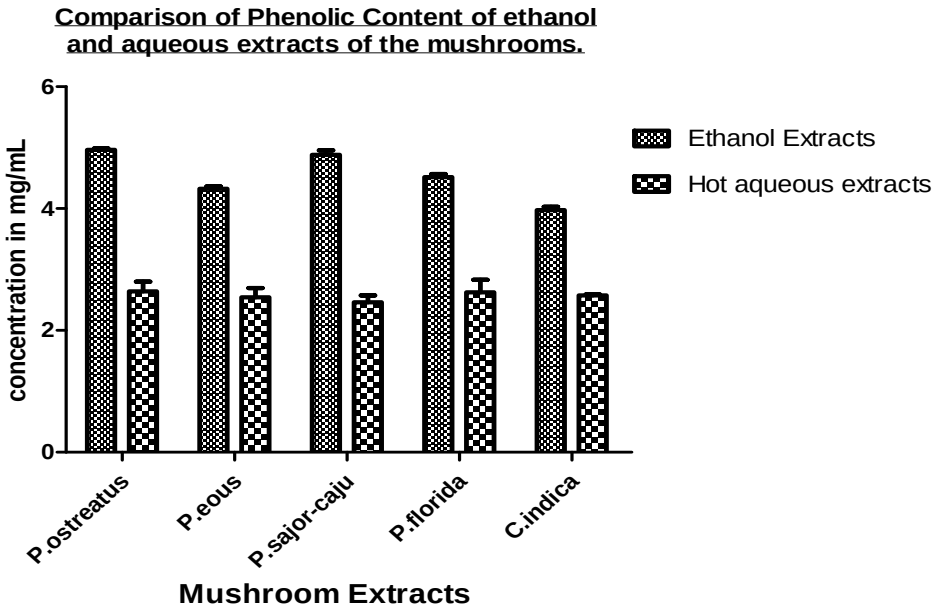

Fig. 1: Graph showing the comparison between total phenol content of the ethanol and hot aqueous extracts of the five edible mushrooms.

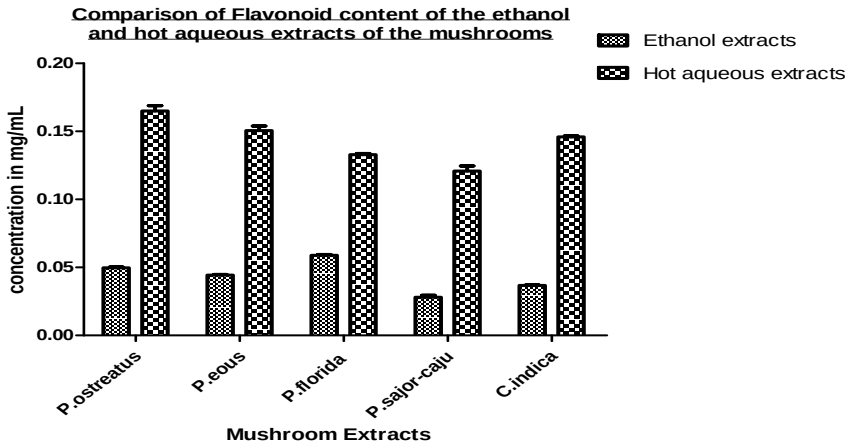

Fig. 2: Graph showing the comparison between the total flavonoid content of the aqueous and ethanol extracts of the five edible mushrooms.

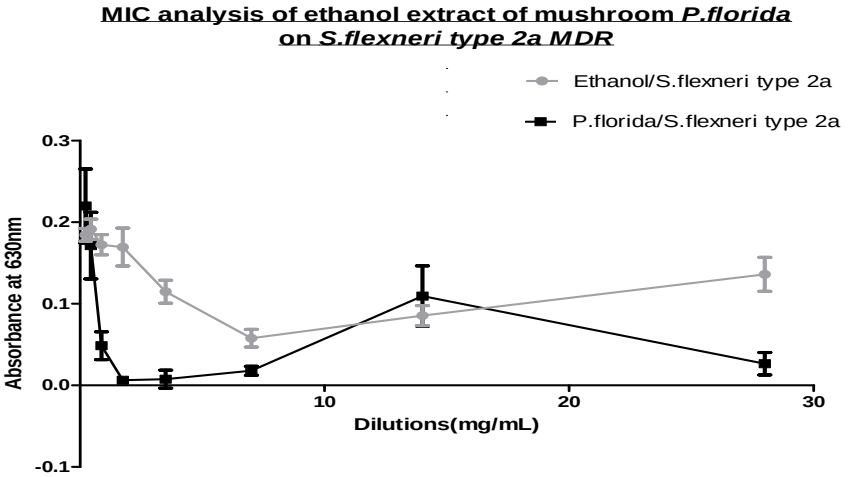

Fig. 3: Graph showing the MIC concentration of ethanol extract of mushroom *P.florida* against multidrug resistant *S.flexneri* type 2a.

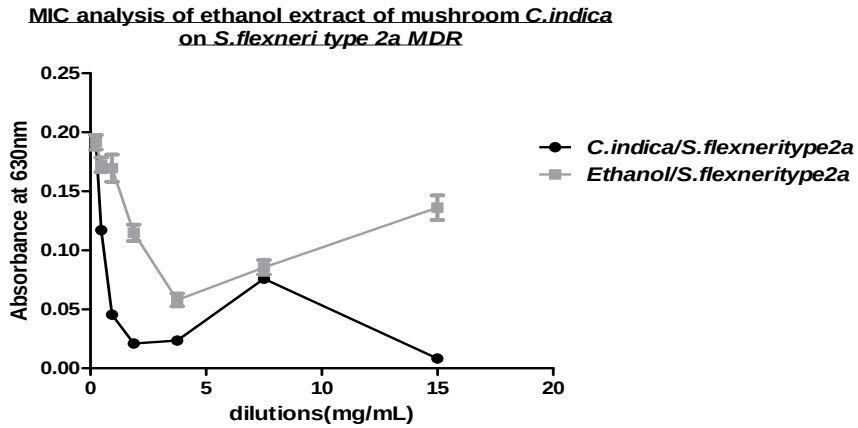

Fig. 4: Graph showing the MIC concentration of ethanol extract of mushroom *C.indica* against multidrug resistant *S.flexneri* type 2a.

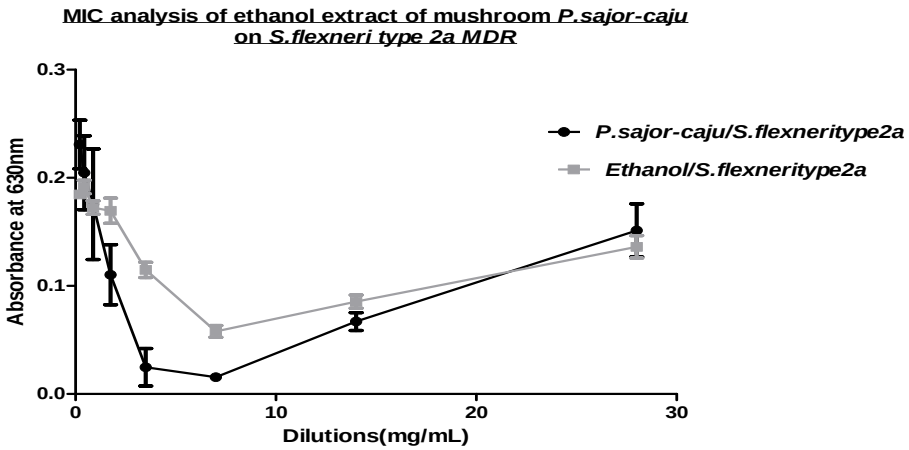

Fig. 5: Graph showing the MIC concentration of ethanol extract of mushroom *P.sajor-caju* against multidrug resistant *S.flexneri* type 2a.

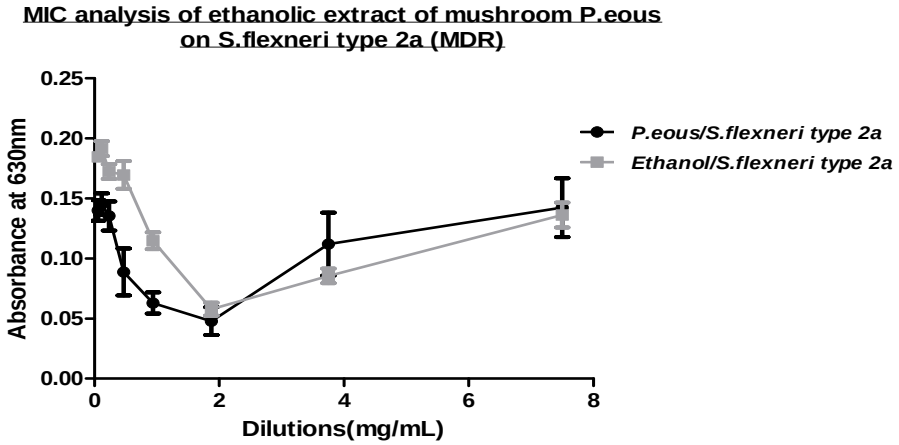

Fig. 6: Graph showing the MIC concentration of ethanol extract of mushroom *P.eous* against multidrug resistant *S.flexneri* type 2a.

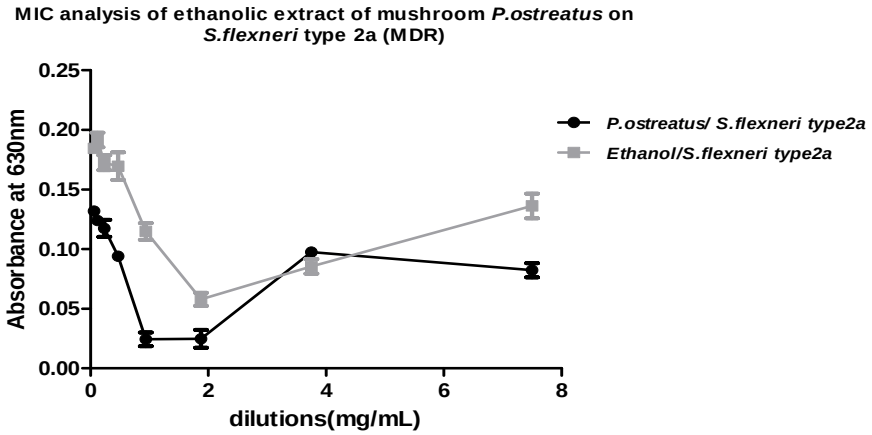

Fig. 7: Graph showing the MIC concentration of ethanol extract of mushroom *P.ostreatus* against multidrug resistant *S.flexneri* type 2a.

DISCUSSION

The results showed promising antimicrobial activity of all the five edible mushrooms against *Shigella* strains including the multidrug resistant strain. The MIC value ranges between 0.94mg/mL – 15 mg/mL. Among all the mushrooms *P.ostreatus* showed best activity against the multidrug resistant strain having the lowest MIC value and *C.indica* showed the highest MIC value of 15mg/mL. The order of the antibacterial activity of mushrooms from highest to lowest is *P.ostreatus*, *P.florida*, *P.eous*, *P.sajor-caju*, *C.indica*. Previous study also revealed antibacterial activity of various organic extracts of the oyster mushroom *P.ostreatus* against many microorganisms but hardly any study mentioned its activity against *Shigella* sp. [12] Few studies revealed about the antimicrobial activity of another oyster mushroom *P.florida* against *Shigella* sp. [2] The ethanol extracts showed antibacterial activity probably due to the higher extraction of the secondary metabolite phenol known for its antimicrobial activity. The difference was statistically significant showing P value < 0.0001 by Two-Way ANOVA analysis with GRAPHPAD PRISM Version 5.

CONCLUSION: This study revealed potent antimicrobial activity of alcoholic extracts of five edible mushrooms of genus *Pleurotus* and *Calocybe* namely *P.ostreatus*, *Pflorida*, *Peous*, *P.sajor-caju* and *C.indica* against *Shigella* spp.

ACKNOWLEDGEMENT

The authors acknowledge Ram Krishna Mission mushroom cultivation farm, Narendrapur for providing mushroom samples. The authors also acknowledge NICED, Kolkata for providing the multidrug resistant microorganisms for the experiments and we would also like to acknowledge DST INSPIRE Fellowship division for providing the financial assistance throughout the work.

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