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Evaluation of Phytochemicals from *Malbranchea cinnamomea*, a thermophilic fungi isolated from compost (vegetable waste compost)

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Abstract- The study was conducted at Department of Botany, Bangalore University, Bengaluru. Vegetable wastes are created during harvesting, transportation, storage, marketing and processing. The mixed vegetable waste were collected from different markets and then composted in heaps and pits. Composting was done during the year 2013. The temperature of the compost were recorded and then the soil samples were collected during thermophilic stage in polythene bags and bought to the laboratory. Isolation was done on Potato Dextrose Agar media (PDA) following serial dilution method and cultures were purified on PDA. The present study was carried out to evaluate the presence of phytochemicals from the isolated thermophilic fungi namely *Malbranchea cinnamomea*. Then the phytochemicals like totalphenols and flavonoids were estimated or analysed both qualitatively and quantitatively following standard procedure. In conclusion, the culture filtrate of *Malbranchea cinnamomea* has an ability to produce the phytochemicals. Among the phytochemicals the total phenols was maximum than tannins and flavonoids.

Key words: Flavonoids, *Malbranchea cinnamomea*, Thermophilic fungi, Tannins, Total phenol, Vegetable waste compost.

I. INTRODUCTION

Fungi are one among the most widely distributed organisms on earth [1]. Among the eukaryotes, only thermophilic fungi have the exceptional ability to grow at a high temperature 50-60ºC [2]. The thermophilic fungi are those which have the maximum temperature for growth at above 50 ºC and minimum temperature for growth at or above 20 ºC. Their prevalence in the composts is explained on the basis that prolonged elevated temperature humid and aerobic conditions and supply of carbohydrate and nitrogen in the mass of organic matter favours the development of thermophilic microflora.

Many biological materials show active decomposition accompanied by rise in temperature and are considered suitable for composting such as agricultural by products, crop residues, animal wastes, vegetable market wastes, food processing wastes and municipal refuse [3].

During composting, thermophilic, thermotolerant and mesophilic micro-organisms decompose cellulose, hemicelluloses and lignin of substrates [4]. The composting process is an exothermal biological oxidation of organic matter carried out by the microbial populations or micro-organisms.The heterogenous organic matter of the raw material is transformed after a suitable composting period into a stabilized end-product through partial mineralization and humification [5].

Composting is the biological conversion of solid organic waste into usable end products such as fertilizers, substrates for mushroom production and biogas. High organic matter content and biological activity make composts effective in a variety of applications [6].Fungi affect soil fertility, suppress plant diseases and promote mushroom growth [7].

Malbranchea cinnamomea is a thermophilic fungi isolated from market waste compost. Furthermore, the fungi are able to produce some phytochemicals.

II. MATERIALS AND METHODS

Isolation

Vegetable wastes (Cabbage, tomato and mixed vegetable) are collected from different markets in and around Bangalore and were brought to the Department of Botany Bangalore University Bangalore and composted in heaps and pits. The samples were collected during thermophilic state of composting (50-55 ºC) in polythene bagsand used for further process. The dilution plate technique [8]was employed to isolate fungi from vegetable waste composts. The thermophilic fungi were isolated on Potato Dextrose Agar media(PDA).

Identification

The culture were identified from Microbial Type Culture Collection Centre (MTCC, Chandigarh) as *Malbrancheacinnamomea* and deposited with reference number **MTCC 12145**.

Culture characteristics.

The genus *Malbranchea*, has a sclerotial conidioma, conidiophores reduced, hyaline, conidiogenous cells, thallic, arthic, conidia hyaline, in coiled chains, 1-celled.

Malbranchea cinnamomea is thermophilic, colonies appeared robust, almost filling the petridish, dense, thick, smooth, velvety, with coarse, creamy yellow tufts of hyphae. The colour is sulphur yellow with yellow to pink margin. Large deposits of dark brown exudates present, the medium turns dark brown to black. The arthroconidia are borne as curved or loosely coiled lateral branches arising from the broader vegetative hyphae, the conidia are cylindrical, often curved, thick walled, hyaline at first, later yellow 2.5-3.5 µm diameter.

Maintainence of culture

The mother cultures were maintained on Potato Dextrose Agar (PDA) slants and stored at 4 ºC and they were sub cultured once in 6 to 8 weeks.

Figure-1: Growth of *Malbranchea cinnamomea* on PDA media.

Phenols

The extract is dissolved in 5ml of distilled. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds.

Tannins

The fungal crude extract was treated with alcoholic Ferric chloride reagent. The bluish colour disappeared on addition of dilute sulphuric acid followed by the formation of yellowish brown precipitate indicated the presence of tannins.

Saponins

The presence of saponins were determined by froth test. The crude dry powder of the fungal extract was vigorously shaken with distilled water and was allowed to stand for 10 minutes. Appearance of no froth indicated the absence of saponins and stable froth indicated the presence of saponins.

Cultivation and extraction of mycelia of fungi.

Fungi were grown on PDA at 55 ºC for 7 days. The 250ml Erlenmeyer flask containing 100ml of liquid PDB (potato dextrose broth) medium were sterilized for 15 minutes at 121º C. The media were inoculated with *Malbranchea cinnamomea* and incubated at 55 ºCfor 5 days. After the completion of incubation, mycelia are separated from the liquid medium by filtration and drying at 50 ºC. The dry mycelium was pulverized and extracted with ethanol (1:1(V/V)) three times. The supernatant was separated by centrifugation at 5000rpm for 10 minutes, fractions were pooled and ethanolic extract was concentrated under reduced pressure to yield the final extract. Alcoholic extract of the fungal species were stored in dark at 4 º C before being used for the bioactivity test.

Prelimnary qualitative phytochemicals screening

The alcoholic extract of *Malbranchea cinnamomea* were checked or screened for the presence of the following secondary metabolites such as alkaloids, phenols, flavonoids, tannins and saponins by standard procedures [9, 10].

Quantitative estimation of phytochemicals

Total Phenolic content

The total phenolic contents in the extract were determined according to the Folin-Ciocalteu method of [11]. To 1 ml of ethanolic extracts, 2ml of 7.5% (W/V) sodium carbonate solution was added and vortexed vigorously. After 5 min,

1ml of 1:10 diluted folin-ciocalteu phenol reagent was added and vortexed again. Procedure for above was followed for the standard solution of gallic acid. The test tubes were incubated at room temperature for 30 minutes and the absorbance was measured at 765 nm by using spectrophotometer (Elico). The total phenolic content in the extracts were expressed as gallic acid equivalent in mg/g (GAE mg/g extract).

Total Flavonoid content

Total flavonoid content was determined according to [12]. The fungal extract (250 µl) was mixed with distilled water (1.25 ml) and Sodium nitrate solution (5%, 75 µl). After 5 minutes the $AlCl_3$ solution (10% 150 µl) was added to the mixture. The solution was mixed well and the intensity of the pink colour was measured at 510nm against blank. The content of the flavanoid was calculated on the basis of the calibration curve of quercetin and the results were expressed as mg of quercetin equivalents per g of extract.

Tannins

The Tannin content was determined by [13]. 1 ml of different aliquots of sample or standard, 5 ml of Folin denis reagent was added, 10 ml of sodium carbonate solution and diluted to 10 ml with distilled water and shaked well and read at 700 nm after 30 minutes against reagent as blank. The content of tannins was calculated on the basis of the calibration curve of tannic acid and the results were expressed as mg of tannic acid equivalent to per g of extract.

III. RESULTS

The total phenol content of fungal crude extract was found to be 4.98mg GAE/g.

The total flavonoid content of the fungi was found to be 3.54 mg Quercetin Equivalent/ g of extract.

The tannin content of fungi was found to be 4.78 mg/g Tannic acid.

IV. DISCUSSION

In this study the thermophilic fungi were isolated from vegetable waste compost. *Malbranchea cinnamomea* were selected as the result shown was good. In the present study the fungi was identified as *Malbranchea cinnamomea* by MTCC.

The phytochemical analysis was carried out for the identified *Malbranchea cinnamomea*. The *Malbranchea cinnamomea* showed the presence of different phytochemicals such as phenolic compounds, tannins, alkaloids and flavonoids.

There are many bioactive compounds observed in the *Penicillium* sp. Our results are similar to the available reports of Nameirakpam Nirjanta Devi *et al*. 2012 [14].

Major natural products of secondary metabolism in plants and fungi are phenolic compounds. Phenol and flavonoid compounds have been reported to possess different bioactivities [15].

Alcoholic extract of *Malbranchea cinnamomea* showed higher phenolic and flavonoid content. It was found that total phenolic content of *Penicillium granulatum* was 7.01mg GAE/g, while in endophytic *Penicillium* sp was only 1mg GAE/g [16, 17].

V. CONCLUSION

Malbranchea cinnamomea has an ability to produce various secondary metabolites which will be useful for various purposes. So it could be recommended as an organism of pharmaceutical importance.

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