# بسم الله الرحمن الرحيم

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تم رفع هذه الرسالة بواسطة / سلوي محمود عقل

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# MICROBIAL PRODUCTION OF TANNASE USING AGRO-INDUSTRIAL WASTES

By

**SARA ASHRAF ABD-ELMOTEY ABD-ELAZEM**
B.Sc. Agric. Sc. (Agric. Microbiology), Fac. Agric., Ain Shams University, 2015

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**Ain Shams University**

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**Approval sheet**

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# ABSTRACT

**Sara Ashraf Abd-Elmotey: "Microbial Production of Tannase using Agro-Industrial Wastes". Unpublished M.Sc. Thesis, Department of Agric. Microbiology, Faculty of Agriculture, Ain shams University, 2022.**

In this work, microbial sources can be used in the biotechnological synthesis of tannase. Microbial tannases are preferred because they are more stable and produced in higher yields than similar ones acquired from other sources. In addition, they can be exposed to genetic manipulation more easily than plants and animals. Tannase has a wide range of industrial uses, including food, and environmental biotechnology. A total, 255 isolates (16, 78, and 161 isolates of yeast, bacteria, and fungi), respectively were obtained from different sources of food, soil, and seawater). Only 10 fungal isolates among 255 gave the highest tannase index on solid medium supplemented with tannic acid as a sole carbon source. Two fungal isolates of T11 and SWP33 were selected which appeared a high significant tannase (122.2 and 127.6 U/ml), gallic acid production (255.4 and 238.4 mg/ml) and the tannin degradation percentage reached to 89.8 and 88.7%, respectively. Both isolates were identified based on morphological, cultural characters, and further confirmation by sequencing the 18S rRNA gene and was identified as *Aspergillus niger* ok 626231 (SWP33) and *Penicillium griseoroseum* ok 626651 (T11). Maximum tannase (147.0 and 148.7 U/ml) and GAC (255 and 258 mg/ml) production by *A. niger* SWP33 and *P. griseoroseum* T11 in the presence of tannic acid was attained on the fourth and fifth days of fermentation, with specific enzyme and GA rates of 0.0332 and 0.0274 $h^{-1}$ and 0.0089 and 0.0099 $h^{-1}$, respectively. The tested strains were cultivated on tannins-rich wastes as a low-cost medium for tannase production using submerged and solid-state cultures techniques. Results indicated that the solid-state fermentation was more preferred for tannase (~40 and ~25 % over increase) and gallic acid (~39

and ~22 % over increase) production than submerged fermentation by *A. niger* SWP33 and *P. griseoroseum* T11, respectively. Tannase and gallic acid was maximized using statistical experimental three- steps approach response surface methodology (RSM). First, screening the best tannin substrate as a sole carbon source (banana peels) and nitrogen source (urea), second evaluation of environmental variables and selected the most significant factors, and third application of complex sequential surface methodology for further optimization using Hybrid design. The enzyme produced by mixed cultures was partial purified with acetone (99%), which it was purified 1.2-fold more thoroughly. Some application for juice clarification, dyes decolorization, and as antitumor were tried. During 6 hours of incubation, fungal tannase demonstrated activity in decolorizing reactive blue 19 and red 24 textile dyes. Tannase was able to the clarifying fruit juices of orange, strawberry, apple, guava, pomegranate, and grape after incubated at 28°C for 2 h with agitation at 150 rpm using a rotary shaker. Antitumor: intestinal carcinoma cells were used for studying the antitumor effect of tannase. The half-maximal inhibitory concentration $IC_{50}$ was calculated to be 2.15 ± 0.19 µL/ mL. The enzyme was assumed that the enzyme had interacted with the cell membrane, causing complete cell membrane destruction.

**Keywords:** Tannase production, Gallic acid synthesis, 18S rRNA sequencing, Partial purification, Solid state-fermentation, Submerged fermentation, Tannase applications, Rich tannin substrates, Factorial design, Response surface methodology, and Dye decolorization.

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