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Research Article

Improvement of paromomycin production by *Streptomyces rimosus* subsp *paromomycinus* NRRL 2455 using gamma irradiation mutagenesis

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ABSTRACT

Streptomyces (S.) rimosus NRRL 2455 produces paromomycin, a 2-deoxystreptamine aminocyclitol aminoglycoside antibiotic (2DOS-ACAGA) with broad-spectrum activity against most of the Gram-positive and Gram-negative bacteria as well as protozoa. The mutation has become one of the beneficial methods used in enhancing the microbial production. Improvement of the paromomycin production by *S. rimosus* NRRL 2455 was achieved via irradiation mutagenesis using gamma (ɣ) radiation. The culture of *S. rimosus* was irradiated with different doses of gamma radiation (3, 4 and 5 KiloGray (KGy) to find out the best dose for the mutation that gave 99.99% killing. The optimum dose was found to be 4 KGy. Six morphologically changed colony types appeared on tryptic soy agar plates. These colonies were bio-assayed for their antimicrobial activity against standard *Staphylococcus aureus* ATCC 25923 using agar well diffusion technique. A mutant coded 5M showed about 1.44, and 2 fold increase in its activity as compared with the wild-type when cultivated in basal culture or optimized media (soybean meal 30 g/L, NH_4CL 4 g/L, $CaCO_3$ 5 g/L and glycerol 40 ml/L), respectively. Moreover, high genetic stability was observed upon subsequent culturing of 5M-mutant. Therefore, *S. rimosus* mutant-5M can be used as a potential industrial strain for paromomycin production.

Keywords: *Paromomycin, Streptomyces rimosus,* gamma mutagenesis, 2DOS-ACAGA.

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1. INTRODUCTION

Streptomyces is a member of the family Actinomycetaceae. It is characterized by having mycelia and spores at the aerial hypha. They are attributed to the production of secondary metabolites such as antibiotics **[1]**. *S. rimosus* NRRL-2455 produces paromomycin, a 2 deoxystreptamine (2DOS)-containing aminoglycoside antibiotic with broad-spectrum activity against Gram-negative bacteria and most of the Gram-positive bacteria especially *Staphylococcus* strains particularly those resistant to oxytetracycline, erythromycin or carbomycin **[2,3]**. Members of *Streptomyces* display genetic instability, intra-strain morphological differences and there is also a correlation between colony morphology and antibiotic activity **[4]**. Random mutagenesis is considered as an effective way to improve the productivity of industrial microbial cultures **[5]**. It was found that some mutants with altered colony morphology and resulting from basic genetic studies exhibited enhanced activities **[6]**. Also, the change in the medium composition affects greatly the biosynthesis of the produced antibiotic from streptomycetes **[7]**. The current study aimed at enhancing the

infections, cough, gastric ulcers, skin diseases and sore throats. Many pharmacological activities were reported for different *Terminalia* species including antioxidant **[6]**, hepatoprotective **[7]**, antihyperlipidemic, antidiabetic **[8]**, anti-inflammatory **[9]** and cytotoxic activities **[10]**. The plants of the genus *Terminalia* are sources of diverse secondary metabolites, including triterpenes, flavonoids, tannins, and other phenolic compounds **[4, 6]**.

The main objective of this study was to determine the phytochemical constituents of powdered leaves of *T. muelleri*, as well as investigate the free radical scavenging activity of *T. muelleri* leaf extract.

2. MATERIALS AND METHODS

2.1 Chemicals

2, 2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) and L-ascorbic acid (Vitamin C) obtained from Sigma-Aldrich GmbH, Darmstadt, Germany.

2.2. Plant material

Fresh leaves of *Terminalia muelleri Benth*. (Combretaceae) were collected in November 2012 from trees grown in the Zoo Garden, Giza, Egypt. The plant was kindly authenticated by Eng. Therese Labib, the consultant at Orman Botanical Garden, Giza, and National Gene Bank at the Ministry of Agriculture, Egypt. A voucher specimen of *T. muelleri Benth* was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt (ASU TMC2012).

2.3. Extract preparation

The air-dried powdered leaves of *T. muelleri* were extracted with 80% aqueous MeOH. The total extract was concentrated and freeze-dried to obtain a dry powder, then defatted with petroleum ether. The remaining part was concentrated and freeze-dried to obtain a dry powder, which was dissolved in absolute EtOH. The EtOH-soluble portion was then concentrated and freeze-dried to obtain the total extract dry powder abbreviated as (TMEF).

2.4. Methods for phytochemical screening

Powdered leaves of *T. muelleri* were screened for the following phytoconstituents: carbohydrates and/ or glycosides, flavonoids, sterols and/ or triterpenes, saponins, tannins, alkaloids, and anthraquinones according to the standard procedures **[11, 12]**.

2.5. Methods for $DPPH^{\bullet}$ radical scavenging assay

The antioxidant effect of the TMEF and pet. ether-fraction was measured using 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging assay, according to the method described by Bourgou, Ksouri **[13]** with slight modification. Tested samples and L-ascorbic acid (standard), both prepared at different concentrations or ethanol in case of control, were added to 0.25 mM freshly prepared $DPPH^{\bullet}$ ethanolic solution. The mixture was slightly shaken and kept in dark for 30 min at room temperature, the absorbance was determined against a blank at 517 nm using a UV Spectrophotometer. All assays were conducted in triplicates.

Percentage inhibition of free radical $DPPH^{\bullet}$ was calculated as follow:

Inhibition % = $[(A_{Control} - A_{Sample}) / A_{Control}] \times 100$.

Where: $A_{Control}$ is the absorbance of the control reaction.

A_{Sample} is the absorbance in the presence of the tested samples.

To calculate the IC50 **[14]** the concentration of the substrate that causes 50% loss of the $DPPH^{\bullet}$ activity (color), different concentrations of the tested samples where used and the percentage inhibition was calculated. The IC_{50} values were calculated according to the equation for Boltzmann sigmoidal concentration-response

curve using the nonlinear regression fitting models (Graph Pad, Prism Version 6, La Jolla, CA, USA).

3. RESULTS AND DISCUSSION

3.1. Results of phytochemical screening

Table 1 Results of phytochemical screening

Constituents	Results
Carbohydrates and/or glycosides	+
Flavonoids	+
Sterols and/or triterpenes	+
Saponins	-
Tannins	+
Alkaloids	-
Anthraquinones	-

(+) present, (-) absent

From the table, it can be concluded that the phytochemical constituents of *T. muelleri* include tannins, flavonoids, sterols and /or triterpenes and carbohydrates and/or glycosides. Also, results revealed that this species most probably doesn't contain alkaloids, anthraquinones, and saponins.

3.2. Results of the antioxidant activity of ethanol-soluble fraction (TMEF)

The IC_{50} (the concentration that inhibits 50% of the absorbance of $DPPH^{\bullet}$), was determined from the graph plotted for the % inhibition against the concentration **(Fig. 1)**. TMEF IC_{50} was 2.7 µg/mL compared to 60 µM ≈ 10.5 µg/mL for ascorbic acid.

It could be concluded from the obtained values that the ethanol-soluble fraction (TMEF) of *T. muelleri* leaf extract showed a more potent antioxidant activity when compared to the standard ascorbic acid.

4. CONCLUSION

This study demonstrated the potent antioxidant activity of *T. muelleri* leaf extract which might be attributed to its high tannins and flavonoids content. The wide use of genus *terminalia* in traditional medicine for the treatment of various diseases may be in part due to their antioxidant potency.

Conflict of Interest

We declare that we have no conflict of interest.

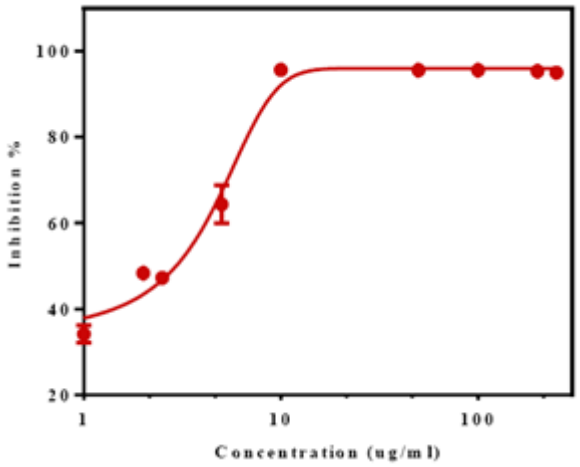

Fig. 1 Antioxidant activity of the TMEF (% inhibition against concentration in µg/ml).

REFERENCES

1. Dasgupta A, K Klein. Chapter 1 - Introduction to Free Radicals and the Body's Antioxidant Defense, in Antioxidants in Food, Vitamins, and Supplements. San Diego: Elsevier; 2014, p. 1-18.

2. Rice-Evans CA, NJ Miller, G Paganga. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med 1996; 20: 933-56.

3. Mukhopadhyay AK. Antioxidants-Natural and Synthetic. Amani Int'l Publishers; 2007.

4. Eloff JN, DR Katerere, LJ McGaw. The biological activity and chemistry of the southern African Combretaceae. J Ethnopharmacol 2008; 119: 686-99.

5. Fahmy NM, Al-Sayed E, Abdel-Daim MM, Karonen M, Singab A. Protective effect of Terminalia muelleri against carbon tetrachloride-induced hepato and nephrotoxicity in mice and characterization of its bioactive constituents. Pharm Biol 2015; 1-11.

6. Pfundstein B, El Desouky SK, Hull WE, Haubner

R, Erben G, Owen RW. Polyphenolic compounds in the fruits of Egyptian medicinal plants (Terminalia bellerica, Terminalia chebula, and Terminalia horrida): Characterization, quantitation, and determination of antioxidant capacities. Phytochemistry 2010; 71: 1132-48.

7. Eesha BR, Mohanbabu AV, Meena KK, Babu S, Vijay M, Lalit, M, et al. Hepatoprotective activity of Terminalia paniculata against paracetamol-induced hepatocellular damage in Wistar albino rats. Asian Pac J Trop Med 2011; 4: 466-9.
8. Latha RCR, P Daisy. Insulin-secretagogue, antihyperlipidemic and other protective effects of gallic acid isolated from Terminalia bellerica Roxb. in streptozotocin-induced diabetic rats. Chem Biol Interact 2011; 189: 112-8.
9. Nair V, S Singh, YK Gupta. Anti-arthritic and disease-modifying activity of Terminalia chebula Retz. in experimental models. J Pharm Pharmacol 2010; 62: 1801-6.
10. Ponou BK, Teponno RB, Ricciutelli M, Quassinti L, Bramucci M, Lupidi G, et al. Dimeric antioxidant and cytotoxic triterpenoid saponins from Terminalia ivorensis A. Chev. Phytochemistry 2010; 71: 2108-15.
11. Harborne J. Phenolic compounds, in Phytochemical methods. Springer; 1973, p. 33-88.
12. Evans WC. Trease and Evans. Pharmacognosy 1989; 33: 471.
13. Bourgou S, Ksouri R, Bellila A, Skandrani I, Falleh H. Phenolic composition and biological activities of Tunisian Nigella sativa L. shoots and roots. Comptes Rendus Biologies 2008; 331: 48-55.
14. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 2004; 26: 211.