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Inhibitory Effect of Extracts from *Datura metel* Leaf on Mushroom Tyrosinase

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Abstract: This study evaluated the tyrosinase-inhibition activities of three extracts; methanol, acetone and dichloromethane of *Datura metel* leaves. The bioactive components were extracted and then evaluated for their inhibitory effect *in vitro* on mushroom tyrosinase, using a colorimetric procedure. The Methanolic extract of the plant leaf had the highest inhibition of tyrosinase conversion of substrate L-DOPA with 72.14% inhibition at peak concentration of 400 µg/ml considered unlike their respective acetone (51.08%) and dichloromethane (65.57%) extracts. All the *Datura metel* extracts had below 50% inhibition between concentrations of 3.1 µg/ml to 200 µg/ml except the methanolic extract which had 53.46% inhibition at 200 µg/ml. Although, there was a steady increase in tyrosinase inhibition for all plant extracts, however none of the plants extracts inhibition exceeded kojic acids inhibition at all concentrations considered.

Keywords: *Datura metel*, Tyrosinase, Inhibition

1. Introduction

It has been assumed that "there is a plant for every need on every continent". Remarkably, this statement appears to be true. Finding healing powers in plants is an ancient idea [1]. The World Health Organization (WHO) estimates that 80% of the people living in developing countries almost exclusively use traditional medicine. According to the database, 74% of pharmacologically-active plant derived drugs have been discovered after following up on ethnomedical use of the plant [2]. Naturally molecules derived from plant extracts offer a particularly exciting avenue for further research [3].

Along human history, people have been struggling with numerous skin diseases, especially skin pigmentation (hyper/hypo). It is also well documented that tyrosinase (EC 1.14.18.1) is an essential enzyme in pigment formation in mammalian's body as well as plants, microorganisms and fungi therefore, tyrosinase inhibitors is becoming increasingly important in several industries. The effect of many other plant extracts (oat, walnuts, chamomile, carrot, almonds, cucumber, lavender, mint and sweet violet petals), have been investigated with a number of tyrosinase inhibitors reported from them [4]. It has been extensively reported that many people in African continent are struggling with various type of skin diseases. According to an investigation in Nigeria, allergic skin diseases (24.9%) were the commonest skin disorders. Within the allergic disorders; eczemas or dermatitis were found to be most prevalent followed by follicular (13.7%) and pigmentary disorders (11.1%) [5].

Plate 1. *Datura metel plant.*

Traditional medicine as defined by the World Health Organization is the total combination of knowledge and practices, whether explicable or not used in diagnosing, preventing or eliminating physical, mental and social causes of social causes of diseases and disabilities [6]. In Nigeria, herbal healing is still widely practiced in rural as well as urban areas due to shortages of drugs and insufficient means to visit established medical centres [6]. Herbalism remains a common occupation in most suburban parts of Nigeria and the rest of Africa till date [7] just as it is still popular in China, India [8]. According to Lewis [9], research on medicinal and other useful plants used in indigenous society has been driven by two complementary interests: the use of such information for research in the field of natural sciences, especially with regards to 'new' bioactive natural products derived from plants and the use of plant extracts in primary health care.

Datura metel was first documented in Sanskrit literature by the Arab Physician Avicenna in the 11th century [10]. It has numerous names, as it is found throughout Africa, Asia, America, Australia, and Europe as either a native or an adventitive plant [11]. Some of its common names are raving nightshade, thorn apple, devil's apple, stinkweed, Jimson weed and angels' trumpet [12, 13]. It is popularly called "gegemu" or "ewe ikan" in Yoruba language. Its use has a very wide array because of its hallucinogenic property and it differs from one continent to another. It is also known to have a wide array of uses especially medicinally. The plant has been in use all over the world from historical times. Folk uses include cure for cancer, local analgesic for burns, sedatives in epilepsy, influenza, cough remedy treatment of Asthma, healing of wounds and treatment of acne [14]. It is used as ritualistic herb and for inebriation purposes because of its hallucinogenic effects [14]. This study aims at investigating the tyrosinase inhibitory activity from the selected plant extracts.

2. Materials and Method

2.1. Plant Collection and Extraction

Datura metel was obtained from Oba-Ile area of Akure, and was authenticated at the Department of Crop Science and Pest of the Federal University of Technology Akure, Ondo State. The leaves of the plant were then picked, air-dried and finely grounded into powdery form using electric blender. Thereafter 5g of powdered sample was dissolved in 25 ml of the three solvents considered, which are: methanol, acetone and dichloromethane. Solution obtained was then incubated at 50°C with continuous agitation for 5 hours in a shaking-water bath to allow extraction, before allowing flask to stand till the next day at room temperature. The solvent and its constituent extracts were filtered off, while the residue was re-dissolved with an equal volume of extracting solvent. The procedure was repeated two more times. The clear filtrate obtained after extraction was placed in fume cupboard for solvents to evaporate and obtain dry extracts.

Table 1. *Plant extraction yield.*

Extracts	Sample Quantity (g)	Extract Yield (g)	Extract Yield (%)
D. metel methanol extract	5.0	0.3	6.0
D. metel acetone extract	5.0	0.1	2.0
D. metel dichloromethane extract	5.0	0.1	2.0

2.2. Tyrosinase Inhibition Assay

Extracts/purified compounds were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 20 mg/ml. This extract stock solution was then diluted to 600 µg/ml in 50 mM potassium phosphate buffer (pH 6.5). Kojic acid were used as positive controls. 700 µl of each sample solution of different concentrations (3.1–400 µg/ml) were combined with 300 µl of tyrosinase (50 Units/ml in phosphate buffer, pH 6.5) in triplicate inside test-tubes. After incubation at room temperature for 5min, 1.1 ml of substrate (12mM L-DOPA) were added to each well. Final concentrations of the extract were 3.1, 6.2, 12.5, 25, 50, 100, 200 and 400 µg/ml. Final concentrations of pure compound and positive control were 1.5, 3.1, 6.2, 12.5, 25, 50, 100 and 200 µg/ml. Test-tubes were incubated for 30 min at room temperature, after which optical densities of the test mixtures were then determined at 492 nm using visible spectrophotometer.

The percentage tyrosinase inhibition was calculated as follows:

$$\text{Percentage Inhibition (\%)} = [1 - \frac{(A3 - A4)}{(A1 - A2)}] \text{ X } 100 \qquad (1)$$

A1= A control i.e. Abs of test with L-DOPA but no sample
A2= Blank i.e. Abs of test with no sample or L-DOPA
A3= A sample i.e. Abs of test with sample and L-DOPA
A4= Abs before adding substrate i.e. L-DOPA

3. Results and Discussion

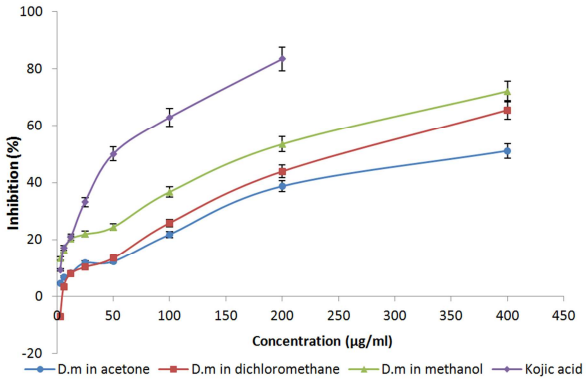

Figure 1. *Percentage tyrosinase inhibition of Datura metel extracts.*

From this study, the oxidation reaction of L-DOPA by

mushroom tyrosinase followed Michaelis-Menten kinetics. The results obtained for inhibition of tyrosinase indicates that the plant extracts of *Datura metel* leaves has inhibitory effect above 50% at highest concentration observed i.e. 400 µg/ml for all extracts. Only methanolic plant extract showed 50% inhibition at a lower concentration of 200 µg/ml (53.46%). The methanolic extract therefore showed the best inhibition of mushroom tyrosinase from studied plant leaves. This result observed confirms similar findings on tyrosinase inhibition of some spices: Black pepper 58.20%, Caraway 58.3%, and Turmeric 88.56%, which showed more than 50% inhibition [15].

Table 2. *IC50 Values.*

Methanol	Acetone	Dichloromethane
175.6 µg/ml	380.2 µg/ml	255.4 µg/ml

The result revealed that all the three extracts exhibit inhibitory effects on tyrosinsae in concentration dependent manner. Tyrosinase inhibitory activities of the methanol, acetone and dichloromethane extracts from the leaves of *Datura metel* increased with increasing concentration. The extracts inhibition ranged from 13.24 to 72.14%, 4.53 to 51.08% and -7.2 to 65.57% respectively. Methanol extract showed a fairly good inhibitory effect on tyrosinase with an IC_{50} of 175.6µg/ml and inhibition of 72.14% at highest concentration considered (400µg/ml). However, acetone extract showed the least inhibitory effect on tyrosinase with an IC_{50} of 380.2µg/ml; while inhibition at highest extract concentration considered was 51.08%. However, at 1.5 to 200 µg/ml, kojic acid; a known tyrosinase inhibitor [4], from this study showed excellent tyrosinase inhibitory activity of 2.53 to 83.48%. The inhibition of tyrosinase might depend on the hydroxyl groups of the phenolic compounds of the mushroom extract that could form a hydrogen bond to the active site of the enzyme, leading to a lower enzymatic activity. Some tyrosinase inhibitors acts through hydroxyl group that bind to the active site on tyrosinase, resulting to steric hindrance or change in conformation [16].

The phytoconstituents such as flavonoids, phenols, tannins, saponins and sterols are found in D. metel [17]. Some other phytochemicals have also been found in D. metel and the main phytoconstituents was reported to include alkaloids [18, 17]. Phytochemical constituents in plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer [19, 20]. All secondary metabolite components displayed antioxidant and antimicrobial properties through different biological mechanisms. Most of the secondary metabolite components were isolated and identified in the polar plant crude extracts [21]. These may be also responsible for the tyrosinase inhibitory activity of *Datura metel* since the phytochemical screening of methanol fresh and dry leaf crude extracts studied showed the presence of active chemical constituents such as alkaloids, flavonoids and saponins.

4. Conclusion

This study has shown that the leaves of the investigated *Datura metel* plant possess considerable level of tyrosinase inhibiting biomolecules especially the methanol extracts which showed the best inhibitory activities. The findings provide scientific basis to promote value-adding of *Datura metel* leaves for further medicinal research purposes.

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