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Research Article

STEAM DISTILLATE OF *MURRAYA KOENIGII* AS A TYROSINASE ACTIVATOR

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ABSTRACT

Tyrosinase (E. C. 1. 14. 18. 1) is ubiquitous enzyme involved in pigmentation that catalyses hydroxylation of monophenol's and oxidation of phenols in presence of molecular oxygen. Deterioration in tyrosinase activity is responsible for vitiligo. In current research we have isolated tyrosinase from germinating jackfruit (*Artocarpus heterophyllus)* seeds and partially purified it using ammonium sulphate (30%) precipitation, and this partially purified enzyme had been used as an enzyme source. Steam distillates of six plants extracts namely *Murraya koenigii***,** *Chrysopogon zizanioides and Phyllanthus emblica , Pandanus odorifer, Santalum album, Rosa rubiginosa* were studied for tyrosinase activation activity. Out of that 100µg of *Vetiveria zizanoides* and *Phyllanthus emblica* increases activity by 2.5 times and *Murraya koenigii* (MK) by 3 times. So extract of MK (curry leaf) were studied in detail. Enzyme kinetic study showed that *Murraya koenigii* is mixed to non-competitive kind of activator. We had further done TLC and active purified constituent used for FTIR study. FTIR structure shows that it contains aromatic amines, activator concentration beneficial to increase tyrosinase activity in different industry. Over all this activator has lots of important in industry and pharmaceutical science.

KEYWORDS: Tyrosinase, *Murraya koenigii,* Vitiligo, Enzyme Kinetics, TLC, FTIR.

INTRODUCTION

Tyrosinase (EC 1. 14. 18. 1) is an oxidant that is the rate limiting enzyme for controlling production melanin synthesis. Melanogenesis is inhibited by down regulation of tyrosinase. Vitiligo is common depigmentation disease difficult to treat. Its prevalence is high in South Asian, Mexican and American population. Inactivation of tyrosinase due to the production of free radical is major reason behind vitiligo. In vitiligo melanocytes did not synthesize melanin in normal conditions but became active to produce melanin under certain stimulation like UV exposure exciter laser and surgical therapy. Steroids also showed benefit but all these therapies reported with long term side effects. In lots of clinical trials many natural health products have been used as medication for vitiligo, like vitamin, minerals and herbal medicine. Plant constituent traditionally used to treat this skin disease. As world wide number of patients is increasing its important to come with better and safe alternatives [19].

Jackfruit seeds common fruit found in *konkan* region though Mushroom is common source of tyrosinase it availability is limited in rainy season so we have preferred jackfruit seeds as tyrosinase source.

The purified tyrosinase from germination jack fruit seeds (*Artocarpus heterophyllus*) was examined for the activator and inhibitor assay which was isolated from *Murraya koenigii* and *Vetiveria zizanoides*, *Phyllanthus emblica*, *Rosa rubiginosa, Santalum album, Pandanus odorifer.* In which *Murraya koenigii* and *Vetiveria zizanoides*, act as activator of tyrosinase enzyme. These plants contain specific aroma and have been reported in many skin cream, face packs, so we have chosen these plant for further study.

MATERIALS AND METHODS

Collection of jackfruit seed

Jackfruit seed (*Artocarpus heterophyllus*) were collected from ripe fruits, location is Ratnagiri, Maharashtra, India. After collection the seeds were cleaned, sun dried and stored for use.

Isolation and Partial purification of Tyrosinase

Extraction of tyrosinase from jackfruit was performed by the method of with few modifications[9], the seeds were cut down into fine pieces and then homogenized (REMI 01-127A) by warring blender at 8000 rpm under cold conditions. 60g of jackfruit seeds were used for enzyme extraction which was carried out in 100ml of cold 0.1mM phosphate buffer (pH 7.0). Extract was centrifuged at 7000 rpm for 20 min and supernatant was collected and used for further enzyme purification.

Clear supernatant was used as enzyme source. This extract was precipitated to 30% with ammonium sulphate followed by centrifugation at 7000 rpm for 20min in cooling centrifuge and then enzyme activity was checked.

Further precipitate was dialysed. Against phosphate buffer pH 7.0 (0.02M) purified enzyme stored at -4^{0}C and used as enzyme source.

Plant extraction method

Six plants *Murraya koenigii (*leaves), *Vetiveriavv zizanioides* (roots) *and Phyllanthus emblica* (fruits) *Pandanus odorifer* (flower), *Santalum album* (stem) *Rosa rubiginosa* (Flower) were used for study. Plant materials were identified from department of botany Gogate Jogalekar College, Ratnagiri. All plant material

were sun dried. 10g plant powder in 100ml Distilled Water used for steam distillation. The distillate treated with Dichloromethane (DCM) (2:1) for solvent extraction. DCM layer was collected and evaporated. The residue was weighted and dissolved in Dimethyl sulphoxide (DMSO),1 mg in 1000µl proportion and use for the further enzymatic studies.

Assay of tyrosinase Activity

The tyrosinase activity assay was performed as reported by Sung and Cho spectrophotometrically measuring conversion of L-DOPA to red colour oxidation product dopachrome (475nm). 1ml of L-DOPA solution (4mg/ml) used as substrate. One unit of tyrosinase activity was defined as enzyme quantity which converts 1µmole of L-DOPA for 1 min. at pH 7.
Total protein contain was determined[10]. Specific activity was 0.053U/mg.
Effects of Different plant extract were studied on this enzyme. Concentration of all extract was 100µg.

Kinetic Analysis

Different Concentrations of MK at different time where used to study activation of enzyme.

To determine type of activator, tyrosinase was incubate with different concentration of substrate L-DOPA in 0.1M phosphate buffer (pH 7.0) at 37^0C for 5 min. inhibition done using 2N NaOH. Absorbance was measured at 475 nm spectrophotometricaly. Similar procedure was repeated with 100µg concentration of *Murraya koenigii*

Km value was determined using Michaelis`-Menten plot and Lineweaver Burk plot[4].

Thin Layer chromatography (TLC)

TLC of *Murraya koenigii* was done using Toluene-ethyl acetate as a mobile phase. Identification of constituent was done in iodine and UV chamber, marked spots were collected into acetone, centrifuge and supernatant was dried dissolved in DSMO and checked activity for tyrosinase. The active component further utilised for the FTIR.

FTIR (Fourier Transform Infrared Spectroscopy)

The active constituent separated by TLC used for FTIR screening (JASCO FT/IR-4100).

Statistical analysis

Values were represented as mean ±SD. Data was analysed with analysis of variance (ANOVA).

RESULT

Screening of plants as tyrosinase activator

Six different plants which have been reported in Ayurveda for different screen treatment selected for tyrosinase activation assay a 100µg concentration of extract used to study enzyme activation.

Santalum album is well known tyrosinase inhibitor (It's inhibiting the tyrosinase 100%).From figure 1 it impels that Steam distillate of *Pandanus odorifer* and *Rosa rubiginosa* don't show any activity on tyrosine. *Murraya koenigii, Vetiveriavv zizanioides and Phyllanthus emblica* act as good tyrosinase activator.

Compare to all plants *Murraya koenigii* acts as a strong activator increasing the activity of enzyme 3 times so for further study were done in presence of only *Murraya koenigii*

Effect of *Murraya koenigii* as an activator at different concentration and at different time interval

From figure 2 it observed that 50µg MK dose not show any kind of activation. Maximum activation is observed after 30 min compare to 10 min and 20 min. 250µg *Murraya koenigii* increases activity 3 times while almost 5 times increased activity observed at 1mg concentration.

Study of Enzyme Kinetic Parameters

To investigate type of activator effect of same concentration of *Murraya koenigii* studied on tyrosinase at different concentration substrate figure 3. The Michaelis`-Menten as well as Line-Weaver burk plot shows *Murraya koenigii* shows mixed to non competitive type of inhibition. In both graph activator is responsible for increasing *Vmax* and decrease in *Km* value

Separation of plant active constituent using TLC

Different solvent systems were used to separate out *Murraya koenigii* constituent by TLC while toluene: ethyl acetate solvent system had given proper separation.

Two spot observed with 0.49 and 0.79 RF value. Both spots screened for tyrosinase activation activity. The spot with RF value 0.49 is major active component. Another spot with RF value 0.79 neither showed activation nor inhibitor of tyrosinase.

FTIR spectroscopy of active constituent separated by TLC

In *Murraya koenigii* fraction with RF value 0.49 screened for FTIR showed the peak at 1752.98 cm^{-1} which shows presence of esters aliphatic. The absorption at 1201.43 cm^{-1} and 1046.19 cm^{-1} is associated with the presence of aliphatic amine. The value of 1360.53 cm^{-1} shows the presence of nitro compound and 676.892 cm is characteristic to the presence of aromatic compound.

DISCUSSION

Tyrosinase play an important role in melanogenesis is over activity of enzyme leading to hyperpigmentation of skin while under activity leads to disorder such as vitiligo. Bacterial tyrosinase used to treat contaminated waste water of a number of industries such as coal conversion resin and plastics, textiles dyes, pulp and papers. Tyrosinase removes toxic phenol. The enzyme also has many applications in food industry. The demand of enzyme has been increased in various industries[21].

This natural enzyme often purified to only a low degree. The plant and human tyrosinase show structural similarities. In current research we have isolated it from Jack fruit seed which gives a good yield from cheap source. In current study different plants initially screened as tyrosinase activator. Steam distillates of *Santalum album*, a well known inhibitor of tyrosinase shows 100% inhibition[3], but *Pandanus odorifer* and *Rosa rubiginosa* unable to show any inhibition activity. May be due to steam distillation it's lost its active constituent which act as tyrosinase inhibitor. *Vetiveriavv zizanioides* and *Phyllanthus emblica* also act as activator but *Murraya koenigii* is stronger one.

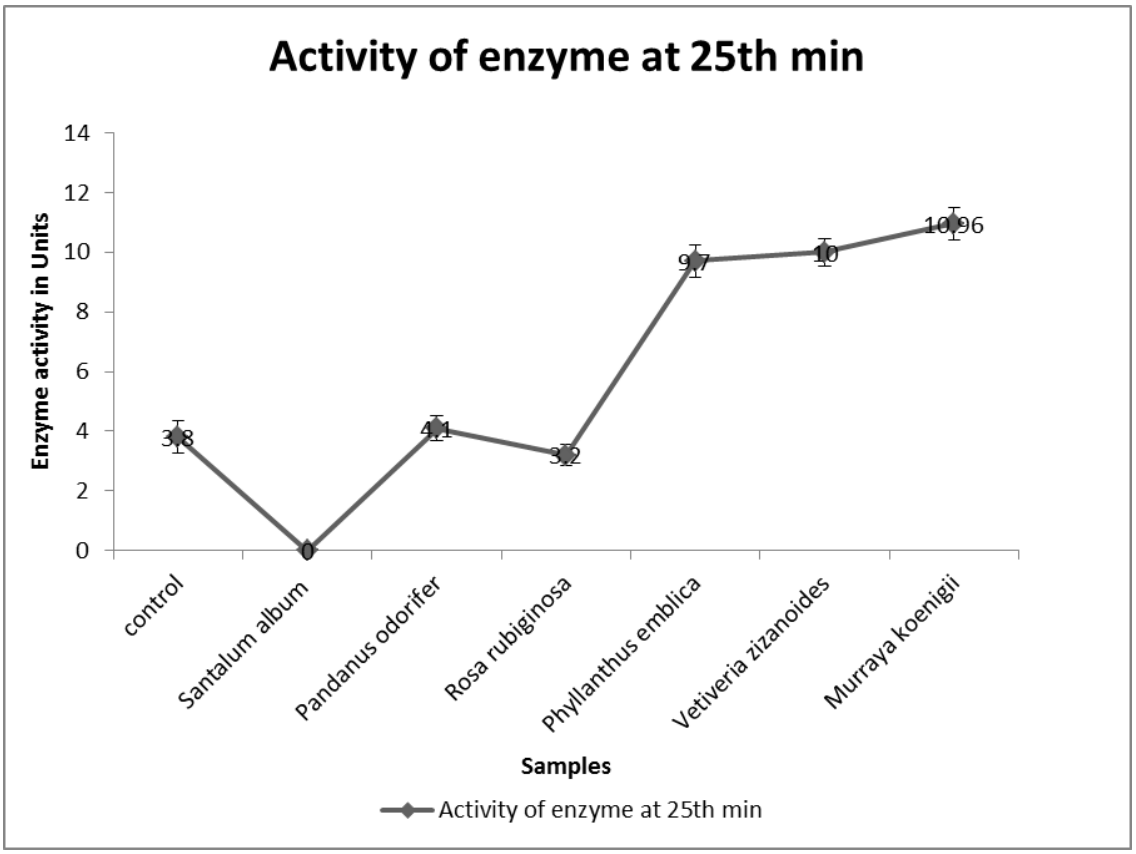

Figure 1:- Tyrosinase activity in presence and absence of plants constituent at 25th min (Activity of plants constituent at 25th min)
◆10 min, ▢20 min, △30min

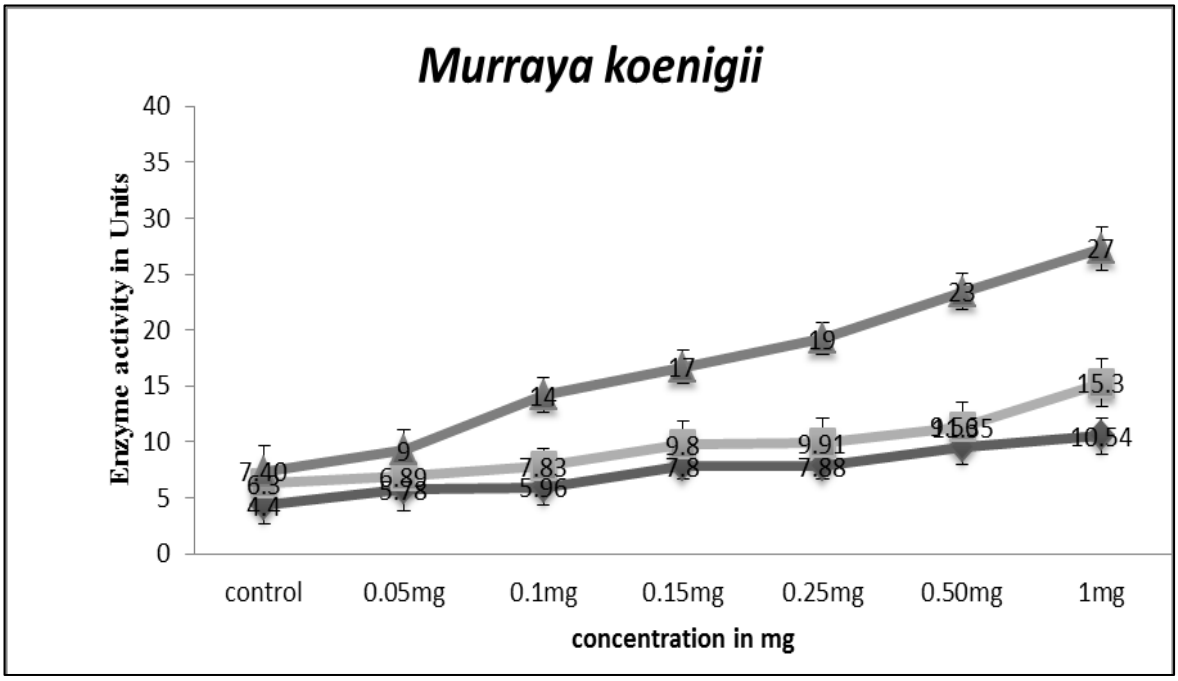

Figure 2:- *Murraya koenigii* as an activator: effect of *Murraya koenigii* on tyrosinase at different concentration and different time interval. (Enzyme activity after ◆10 min, ▢20 min, △30min)

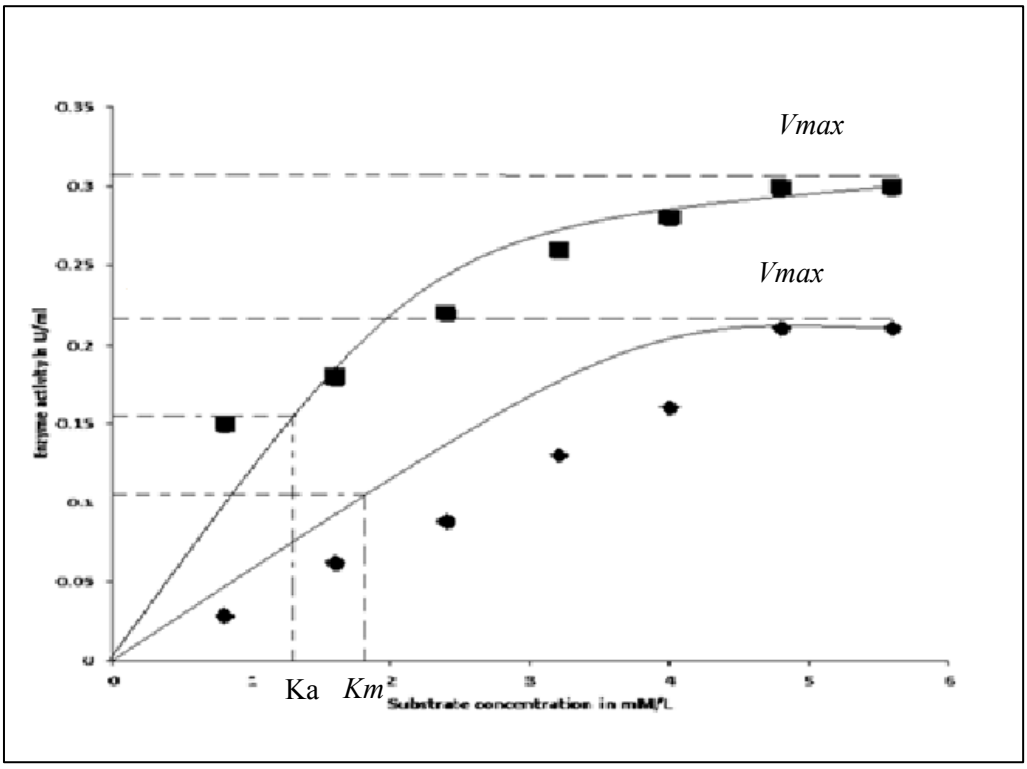

Figure 3:- MM plot of tyrosinase in presence and absence of activator (concentration of Mk 100µg) (Tyrosinase activity without activator, Tyrosinase activity with activator)

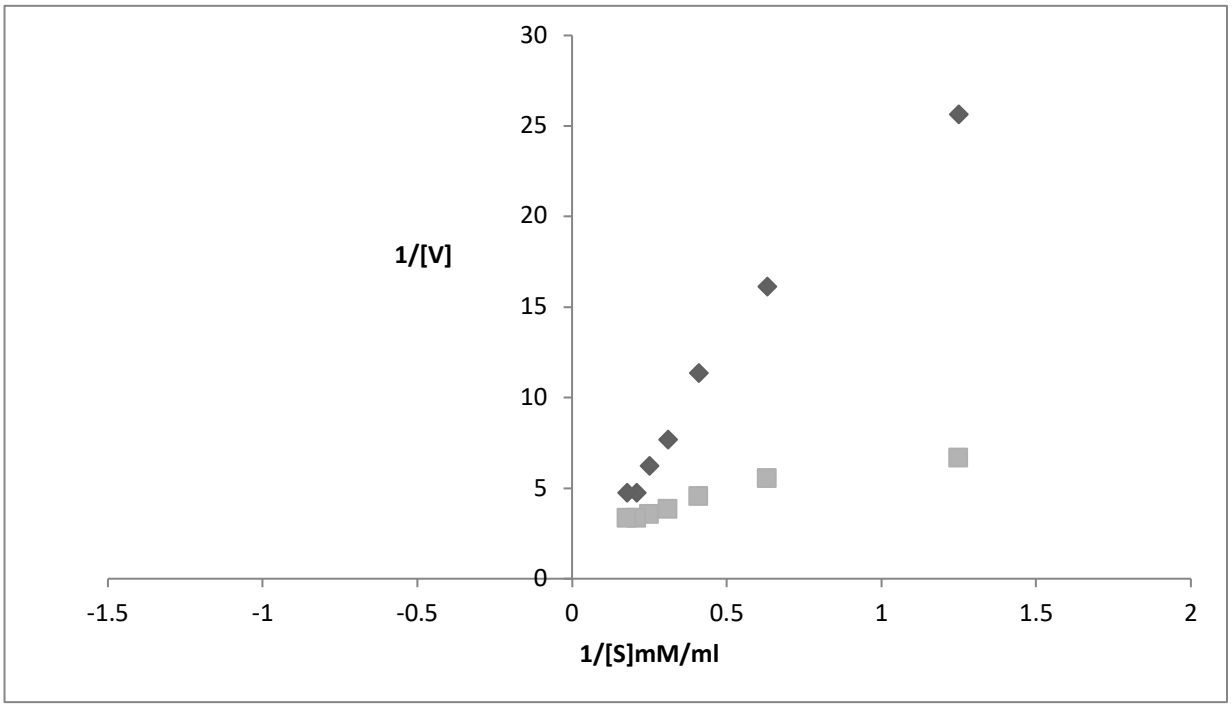

Figure 4: - Line-Weaver burk plot *Artocarpus heterophyllus* with and without *Murraya koenigii.*(concentration of *Murraya koenigii* 100 µg) (Tyrosinase activity without activator, Tyrosinase activity with activator)

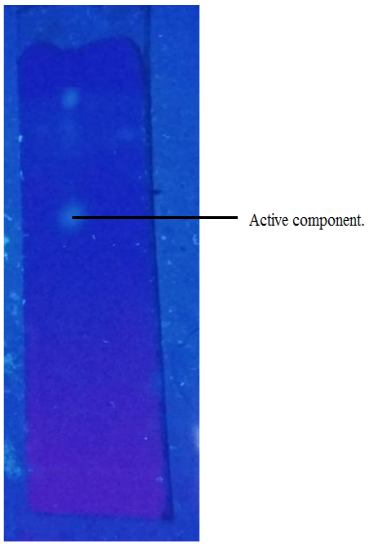

Figure 5: Separation of *Murraya koenigii*s active component using TLC (toluene: ethyl acetate), detection under UV detector.

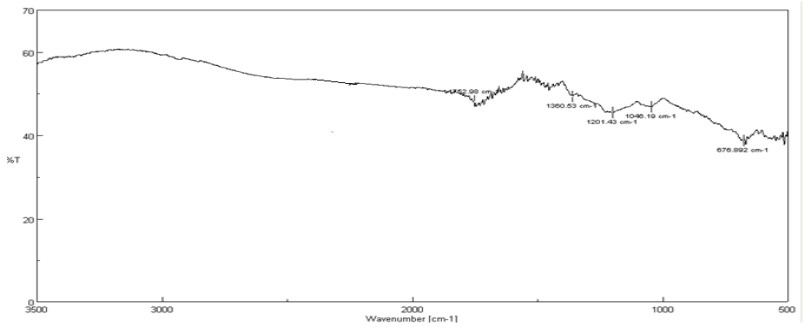

Figure 6: Fourier Transform Infrared Spectroscopy Spectroscopy(FTIR) of active constituent *Murraya koenigii*

Murraya koenigii also reported for antioxidant activity[7]. With time and increased concentration *Murraya koenigii* shows high level of activation of tyrosinase. The activator effect is mixed to non-competitive type. Thus *Murraya koenigii* is affects other than active site of enzyme The purification with TLC and FTIR shows that its basic structure contains aromatic amine. In India curry leaf favourite spice and traditionally used for to treat greying of hair. Activation of tyrosinase may be an answer to vitiligo as in many cases deterioration of tyrosinase activity is responsible for white patches. Skin tanning by UV rays is famous in western countries but it may lead to hazardous side effects, MK can be used such tanning creams. If isolated compound does not show any toxicity it can be used in downstream processes of water purification as well as in food industry to enhance activity of tyrosinase making these processes cheaper.

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