Evaluation of Catalase and Peroxidase Activities of Ricinus Communis (CASTOR SEED)

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Abstract- ***The work was on evaluation of catalase and peroxidase activities of Rucinus communis (castor seed). The research was done using standard analytical procedure. The result showed that for the catalase activity, at 62.5µg/ml concentration of castor seed, the enzyme concentration was 0.25µmol/ml, at 125µg/ml, the enzyme concentration was 0.42µmol/ml, at 250µg/ml, the enzyme concentration was 0.5µmol/ml, at 500µg/ml, the enzyme concentration was 0.7µmol/ml. The result indicated that was a progressive increase as the concentration of the sample increases thus it was concentration dependent. For the peroxidase activity, it showed that at 62.5µg/ml concentration of castor seed, the enzyme concentration was 0.13µmol/ml, at 125µg/ml ,the enzyme concentration was 0.27µmol/ml, at 250µg/ml ,the enzyme concentration was 0.48µmol/ml and at 500µg/ml ,the enzyme concentration was 0.38µmol/ml. This shows that the peroxidase activity was not concentration dependent due to decrease at the highest enzyme concentration.***

I. INTRODUCTION

Medicinal plants represent rich source of antimicrobial agents. Based on WHO reports, more than 80% of the world population relies on traditional medicine for their primary healthcare needs (Varahalarao and Kaladhar, 2012). Medicines from natural products are the growing world-wide interest and act as complementary or alternative medicine to ameliorate many diseases. The inadequate supplies, high cost, side effects with the modern medicines have led to a reawakening of interest in the plants utilization and their products in recent years (Magaji *et al*., 2008).

India stands as the rich source of medicinal plants and their plant products for the development of therapeutic materials have viewed medicinal herbs as an indispensable part of the traditional medicine practised all over the world for its low costs, easy access and ancestral experience (Prabhu *et al,*2008). It is very important to undertake studies relating to screening of the folklore medicinal plants for their proclaimed biological efficacy (Mali *et al*., 2008).

Ricinus communis is a castor plant belongs to spurge family. Its roots, leaves and seed extracts are famous for their medicinal properties. Its cultivation was stopped in the past due to some diseases caused by ricin obtained from *R. communis*. Its cultivation was again started few decades ago because of its high pharmaceutical and agricultural qualities. It shows different activities. It is plant of African origin. There are few areas like temperate regions, subtropical and tropical regions where *Ricinus communis* could be cultivated in bulk (Bolaji *et al.,* 2012). The Leaves of *Ricinus communis* are green, purple or reddish-green, palmately lobed, broad, having 6-12 lobes, with 25-55 cm diameters. It is almost orbicular in shape, margin serrate, having lobes which are oblong linear, acuminate or acute, having lengthy from 2.5-7.5 cm, in width 4-20 cm, cylindrical or slightly flattened towards distal and palmately attached to blade, petiole 10-20 cm long, solid when young, alternate, becomes hollow on maturity, smooth, palmately-divided and 25-55 cm in width. They have lobes which are toothed and oblong **(**Faheem and Moshin, 2018).

Many ailments like inflammation, hyperlipemia, artherosclerosis, osteoporosis, bone resorption, cardiovascular diseases, immune deficiency, central nervous system disorders and cancer have also been effectively treated with the medicinal plants. Severe ill-fated diseases like diabetes have been shown to have significant relief with the plant extracts and their products (Sathishsekar and Subramanian, 2005).In 18th century the investigation on the properties, characteristics, sources and use of

antioxidant compounds especially flavonoids, vitamins, synthetic chemicals, micronutrients and phenolic compounds was started. It has given us the way to rampant use of antioxidant compounds for the purpose of obtaining and preserving the proper human health (Pontis et al., 2014). There are many food supplements and nutraceuticals which contain natural or synthetic antioxidant compounds. There are some problems about using antioxidants because many beliefs of people from ancient times that consider them dangerous. They are common methods for checking *in vitro* antioxidant capacity and nonenzymatic biochemical present in specific phenolic substances and nonphenolic substances to check the activity of antioxidants present in herbs (Ndhala et al., 2010). Antioxidants are good if they are used for short time span but if they are used as long term in human then their effect is controversial and it can be dangerous.

- General Description Castor Bean (*Ricinus communis*)

Ricinus communis, the castor bean or castor oil plant is a species of perennial flowering plant in the *Spurge* family, Euphorbiaceae. It is the sole species in the monotypic genus, Ricinus and sub tribe Ricininae . Ricinus communis is a tropical plant, known as castor bean, that is distributed widely across the world (Eudmar *et al*., 2011). The plant is native of India and cultivated throughout the country in gardens and fields and also grows wild in waste places. Ricinus Communis is indigenous to North eastern tropical Africa. It was already grown for oil in Egypt some 6000 years and spread through Mediterranean, the middle East and India at an early date. It naturalize easily and grows in many areas as a ruderal plant. It occurs across in African continent, from the Atlantic Coast to the Red sea and from Tunisia to South Africa and in the Indian Ocean Islands.

Ricinus communis is a small wooden tree which grows to about 6 meters in height and found in South Africa, India, Brazil, and Russia. Stems of *Ricinus communis* have Anticancer, Antidiabetic and Antiprotozoal activity (Singh *et al,*. 2010). In the Indian system of medicine, the leaf, root and seed oil of this plant have been used for the treatment of the inflammation and liver disorders, hypoglycemic, laxative (Kensa and Syhed, 2011).

Leaves are alternate, curved, cylindrical, purplish petioles, sub peltate, drooping, stipules large, ovate, yellowish, united into a cap enclosing the buds, deciduous, blade 6-8 inches across, palmately cut for three quarters of its depth into 711 lanceolate, acute, coarsely serrate segments, smooth blue green, paler beneath, red and shining when young. Flowers are monoecious, large, arranged on the thick rachis of an oblong, spicate panicle, which is at first terminal but becomes lateral by the growth of an axillary bud beneath it; male flowers shortly stalked, on branched peduncles at the base of the panicle, pedicels articulated about the middle; female flowers sessile, at the upper part; bracts broadly triangular. Fruit is blunt, greenish, deeply-grooved, tricoccus capsule, less than an inch long, with the prominences of the ovary becomes sharp, weak, spreading spines, 3-celled, dehiscing loculicidally and septicidally into 6 valves. Seeds are ovoid, flattened, nearly ⅝ inch long by ¼ broad, smooth, shining, pinkish- grey, prettily mottled with dark brown, caruncle large, subglobular, raphe faintly raised, running down centre of ventral surface, embryo large in axis of the endosperm, cotyledons foliaceous, broadly ovate, with a cordate base, veined. Roots are light in weight almost straight with few rootlets, outer surface dull yellowish brown, nearly smooth but marked with longitudinal wrinkels (Bentley and Trimen, 2007).Seedlings of castor emerge 10-20 days after sowing. The successive formation of branches and inflorescence originates throughout the plant's life. The node at which the first inflorescence originates is a cultivar characteristics. Flowering starts early in the life of castor. Pollen is mainly shed in the morning and pollination is by wind. Castor has high rate of photosynthesis which is attributed to high chlorophyll content in the leaves. Castor will grow on almost all soil types as long as it is well drained and reasonably fertile.

II. METHODOLOGY

Reagents

- Phosphate buffer 0.2m
- Hydrogen peroxide
- Pyrogallol
- Distilled water

Apparatus

- Crucible
- Homogenizer
- Spectrophotometer

- Sample Collection

Ricinus communis was obtained from the local market of Eke Oko, Orumba North L.G.A of Anambra state, Nigeria. It was identified by a taxonomist, Dr S.I. Okeke from the Department of Science laboratory technology, Federal Polytechnic Oko, Anambra state.

- Sample preparation

The hull of the *Ricinus communis* was peeled and the seed was grounded using crucible.

- Assay of Catalase

Principle

The UV absorption of hydrogen peroxide can be measured at 240nm, whose absorbance decreases when degraded by the enzyme catalase. From the decrease in absorbance ,the enzyme activity can be calculated.

- Preparation of Enzyme Sample

A 62.5µg/ml, 125µg/ml, 250µg/ml and 500µg/ml homogenate of the sample was prepared in phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay.

- Procedure

H_2O_2 -Phosphate buffer (3.0ml) was taken in an experimental cuvette, followed by the rapid addition of 40ul of enzyme sample and was mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240nm in a spectrophotometer (GAERenesys 10-S,USA).The enzyme solution containing H_2O_2 -free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05units

- Assay of Peroxidase

Preparation of Enzyme Sample

A 62.5µg/ml, 125µg/ml, 250µg/ml and 500µg/ml homogenate was prepared in 0.1M phosphate buffer (pH 6.5) clarified by centrifugation and the supernatant was used for the assay.

- Procedure

To 3.0ml of pyrogallol solution,0.1ml of the enzyme sample was added and the spectrophotometer was adjusted to read zero at 430nm. To the test cuvette,0.5 ml of H_2O_2 was added and was mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer (Geneseys 10-S,USA).One unit of peroxidase was defined as the change in absorbance/minute at 430nm.

III. RESULTS

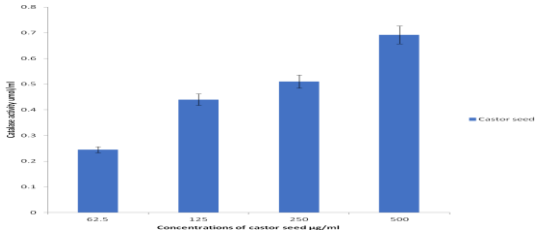

Figure 1. Catalase activities of *Ricinus communis* (castor seed)

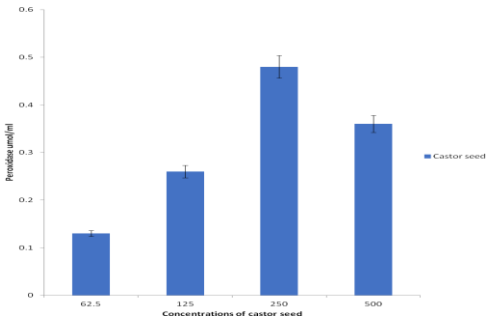

Figure 2. Peroxidase Activities of *Ricinus Communis* (castor seed)

IV. DISCUSSION

Antioxidants are compounds that inhibits oxidation. Catalase and peroxidase are under the primary and enzymatic antioxidants. Catalase is a key enzyme which uses hydrogen peroxide, a non- radical reactive oxygen species (ROS) as its substrate. Its function is neutralisation through decomposition of hydrogen peroxide. For the assay of catalase, at different concentration of the castor seed, the level of

the catalase was the same as the enzyme activity. The result shown in Figure 1 indicated that at the various concentration of the castor seed, that the enzyme concentration or activity varies. At the concentration of 62.5µg/ml, the enzyme concentration was 0.25µmol/ml, at 125µg/ml, the enzyme concentration was 0.42µmol/ml, at 250µg/ml, the enzyme concentration was 0.5µmol/ml and at 500µg/ml, the enzyme concentration was 0.7µmol/ml. The result shows a progressive increase as the concentration of castor seed increases as such the highest enzyme activity or concentration was seen at the highest concentration of castor seed at 500µg/ml. This indicated that the catalase activity of the castor seed was concentration dependent.

Peroxidase is an enzyme that oxidize molecule at the expense of hydrogen peroxide. At different concentration of the castor seed, the level of the peroxidase was the same as the enzyme activity. The result in Figure 2 shows that at different concentrations of castor seed that the enzyme concentration varies such that at 62.5µg/ml of the castor seed concentration, the enzyme concentration was 0.13µmol/ml, at 125µg/ml, the enzyme concentration was 0.27µmol/ml, at 250µg/ml, the enzyme concentration was 0.48µmol/ml and at 500µg/ml, the enzyme concentration was 0.38µmol/ml. The highest enzyme concentration was seen at 250µg/ml concentration of castor seed, at such a decrease in enzyme concentration was seen at the highest castor seed concentration 500µg/ml. Thus, the peroxidase assay of the castor seed was not concentration dependent .

This result agrees with a previous research by Beulah and Ramana (2013) where there was a progressive increase in catalase activities and so was dependent on concentration.

For the peroxidase assay, the result disagrees with a previous work by Hoyle (2006), which showed a result that was concentration dependent. The work also agrees with Saunders (2011), which was not also dependent on concentration.

CONCLUSION

From the findings of the research, it was seen that the catalase activity of the castor seed was dependent on concentration due to its progressive increase as concentration of castor seed increases.The higher the concentration ,the higher the enzyme activity or concentration.

For the peroxidase evaluation, it was seen that the peroxidase activity was not concentration dependent, such that at some point, increase in castor seed concentration lead to a decrease in enzyme concentration.

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