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# *In vitro* antioxidant and anti-diabetic activity of milk yam (*Ipomoea digitata* L.) tubers

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**Abstract**
Milk yam (*Ipomoea digitata* L.) tubers are medicinal having several proven pharmacological properties. In this study, milk yam rooted cuttings were raised in polybags and pharmacological properties viz., *in vitro* antioxidant activity (DPPH radical scavenging activity, nitric oxide radical scavenging activity and reducing power) and *in vitro* anti-diabetic activity (Alpha amalyse inhibition assay) of immature (six months after planting), optimally mature (21 months after planting) and over mature (36 months after planting) tubers were assessed. Pharmacological properties of milk yam tubers found to vary with maturity. DPPH radical scavenging activity and nitric oxide radical scavenging activity of optimally mature milk yam tubers (21 MAP) recorded least $IC_{50}$ values, 1040.00 µg/ ml and 355.00 µg/ ml respectively. Reducing power of optimally mature milk yam tubers observed as absorbance at 400 µg/ ml concentration was 0.118 nm. Ethanolic extract of optimally mature tubers recorded superior *in vitro* anti-diabetic activity (99.20 per cent). This study revealed that, for better pharmacological properties milk yam tubers have to be harvested by 21 months after planting only.

**Keywords:** DPPH, maturity, pharmacological, radical, reducing power

## Introduction
Milk yam (*Ipomoea digitata* L.) is a perennial vine called as Ksheervidhari in Sanskrit. It is a morning glory plant noticed all near the river banks, marshy areas, ponds etc. in tropics. Milk yam tubers have been using in folkloric system and in Indian systems of medicine for its varied pharmacological properties like antioxidant, anti-diabetic, anti-cholesteromic, galactagogic, chalogogic, etc. Milk yam tubers are an important component in many nutraceutical products including Chyavanaprasha and several other ayurvedic formulations [27]. Investigation on pharmacological properties of milk yam tubers revealed its potentiality against different types of life style disorders like diabetes, hypertension, cardio-vascular diseases, infertility, immune deficiency etc. which may be attributed to varied array of nutrients [28] and phytochemicals present in it [25]. *In vitro* antioxidant activity of its tubers was reported [14, 31 21]. *In vivo* antioxidant activity of methanolic extract of the tubers were also reported [1]. 'Umbelliferone' a coumarin derivative with potent pharmacological profile were quantified to be higher in mature milk yam tubers harvested at 21 months after storage [26].
Flavonoids and β-sitosterols in milk yam tubers aided in providing anti-diabetic activity too [35]. Administration of hydroalcoholic extract of milk yam tubers at a dose of 100-200 mg $kg^{-1}$ body weight for 28 days provided significant anti-diabetic activity [16]. Significant reduction in fasting serum glucose, triglyceride and cholesterol levels were recorded in alloxan induced diabetic rats by the administration of methanolic milk yam tuber extract [13]. Chandira and Jayakar [5] had formulated herbal tablets using aqueous extract of *I. digitata* L. and administration of these tablets at a dose of 300 mg $kg^{-1}$ body weight in streptozotocin induced diabetic rats, could significantly decrease the blood glucose level, which indicated its potent anti-diabetic activity.
In this present study, *in vitro* antioxidant activity and *in vitro* anti-diabetic activity of milk yam tubers at different maturity stages was evaluated to find out the ideal stage to harvest the tubers for maximum pharmacological benefits.

## Materials and Methods
The study was performed in the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Kerala Agricultural University ($8^0$ 25' 46'' N latitude & $76^0$ 59' 24'' E longitude) during September 2015- September 2017. A local ecotype of *I. digitata* L. vines were collected from the Instructional Farm, College of Agriculture, Vellayani. A herbarium of *I. digitata* L. was prepared in duplicates and submitted to the internationally recognized Janaki Ammal Herbarium (RRLH) [Accession No.-23207] and authenticated from CSIR-Indian Institute of Integrative Medicine, Jammu.

Uniform rooted cuttings were prepared from two node semi-hard wood cuttings of milk yam vines by raising in polybags. One month old healthy rooted cuttings were then transplanted to polybags (size: 40x24x24 cm and 600-gauge thickness) filled with 1: 1: 1 soil, sand and farm yard manure. Organic management practices and need based irrigation was provided. Sample plants were carefully de-potted at immature (six months after planting), optimally mature (21 months after planting) and over mature (36 months after planting) stages and subjected to pharmacological analysis.

Pharmacological properties viz., *in vitro* antioxidant activity and *in vitro* anti-diabetic activity of optimally mature, immature and over mature milk yam tubers were analysed through standard protocols.

**In vitro Anti-oxidant Activity of Milk Yam Tubers**

**DPPH Radical Scavenging Activity ($IC_{50}$ Value-µg $ml^{-1}$)**

*In vitro* antioxidant activity of milk yam tuber extracts was determined using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The scavenging effect on DPPH free radical was measured according to the procedure of Shimada *et al*. [24]

Ethanolic extract of milk yam tuber powder (2 ml) was added to 0.10 mM DPPH solution (2 ml). The mixture was shaken and left for 30 minutes at room temperature. The absorbance was read at 517 nm. Scavenging effect was expressed as inhibition per cent of DPPH radicals as shown in the following equation:

$$\text{Inhibition per cent} = [(A_{blank} - A_{sample}) \times 100] \div A_{blank}$$

where,

$A_{blank}$ - Absorbance of DPPH solution without sample, read against ethanol blank

$A_{sample}$ – Absorbance of the test sample

The $IC_{50}$ value of the milk yam tuber extracts (the volume of extract required to scavenge 50 per cent free radicals) were calculated using linear regression analysis. Lower $IC_{50}$ value indicates greater antioxidant activity.

**Nitric Oxide Radical Scavenging Activity ($IC_{50}$ Value-µg $ml^{-1}$)**

*In vitro* antioxidant activity of methanolic milk yam tuber extracts was determined by nitric oxide radical scavenging activity as per Sreejayan and Rao [29]. Sodium nitroprusside solution was added to milk yam tuber extracts for the production of nitric oxide free radicals and it was measured by Griess reagent. The ability of the extracts to oxidise nitric oxide radicals and converting it to nitrite ions were assessed by recording the absorbance at 546 nm against a blank. Percentage inhibition of free radicals were calculated as,

$$\text{Inhibition per cent} = [(A_{blank} - A_{sample}) \times 100] \div A_{blank}$$

where,

$A_{blank}$ - Absorbance of solution without sample, read against methanol blank

$A_{sample}$ – Absorbance of the test sample

The $IC_{50}$ value of the milk yam tuber extracts were calculated using regression analysis and compared with standard ascorbic acid. Lower $IC_{50}$ value indicates greater antioxidant activity.

**Reducing Power (nm)**

Reducing power of milk yam tuber extracts were determined by the method of Yildrim *et al*. [36] The supernatant obtained by centrifugation of tuber extract treated with sodium phosphate buffer, potassium ferric cyanide and trichloro acetic acid were diluted, ferric chloride solution was added and absorbance was read at 700 nm. Higher absorbance indicated greater reducing power and standard ascorbic acid was used for comparing the results.

***In vitro* Anti-diabetic Activity**

Anti-diabetic activity of milk yam tubers harvested at different maturity stages [immature (six MAP), optimally mature and over mature (36 MAP)] were evaluated and compared for its anti-diabetic activity using alpha amylase enzyme inhibition assay [7].

**Alpha amalyase enzyme inhibition activity assay (per cent)**

Milk yam tuber powder extracts were added with phosphate buffer (pH 6.9) and treated with alpha amylase (0.50 mg $ml^{-1}$). It was again treated with reaction mixture containing one per cent starch solution and 0.02 M sodium phosphate buffer. The reaction was stopped by adding one millilitre dinitrosalicylic acid (DNS) and keeping the reaction mixture in boiling water bath for five minutes. DNS reagent was added to standard maltose as well and placed in water bath for 15 minutes. To both the standard maltose and the sample 8 ml distilled water was added and the absorbance was read at 570 nm. Amount of maltose produced was calculated using standard maltose curve and enzyme activity was calculated using the given formula.

$$\text{Enzyme activity (per cent)} = (\text{Amount of maltose formed} \times 2) \div (10 \times 342)$$

**Results**

***In vitro* Antioxidant Activity ($IC_{50}$ value-µg $ml^{-1}$, Reducing power-nm)**

Antioxidant activity measured in terms of DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging activity (Table 1), nitric oxide radical scavenging activity (Table 2) were recorded as $IC_{50}$ values (µg $ml^{-1}$) and reducing power recorded as absorbance (nm) (Table 3).

Optimally mature milk yam tubers (21 MAP) recorded significantly least $IC_{50}$ value for scavenging DPPH radicals, 1040.00 µg $ml^{-1}$ and the highest being recorded by immature tubers of six MAP (1858.00 µg $ml^{-1}$). $IC_{50}$ value for scavenging nitric oxide radicals showed least value for over mature milk yam tubers, 314.00 µg $ml^{-1}$ which was on par with optimally mature tubers (355.00 µg $ml^{-1}$). Highest $IC_{50}$ value for nitric oxide radical scavenging activity was recorded by immature tubers (six MAP), 1374.00 µg $ml^{-1}$.

**Table 1:** DPPH radical scavenging activity of milk yam (*Ipomoea digitata* L.) tubers at different maturity stages

| Samples | DPPH radical scavenging activity [$IC_{50}$ value (µg $ml^{-1}$)] |
|---|---|
| Immature tubers (6MAP) | 1858.00 |
| Mature tubers (21MAP) | 1040.00 |
| Over mature tubers (36MAP) | 1193.00 |
| S.Em ± | 19.57 |
| CD (P=0.05) | 60.41 |

**Table 2:** Nitric oxide radical scavenging activity of milk yam (*Ipomoea digitata* L.) tubers at different maturity stages

| Samples | Nitric oxide radical scavenging activity [$IC_{50}$ value (µg $ml^{-1}$)] |
|---|---|
| Immature tubers (6MAP) | 1374.00 |
| Mature tubers (21MAP) | 355.00 |
| Over mature tubers (36MAP) | 314.00 |
| S.Em ± | 13.96 |
| CD (P=0.05) | 43.01 |

**Table 3:** Reducing power of milk yam (*Ipomoea digitata* L.) tubers at different maturity stages

| Samples | Reducing power Absorbance (nm) | | | |
|---|---|---|---|---|
| | 50 µg $ml^{-1}$ | 100 µg $ml^{-1}$ | 200 µg $ml^{-1}$ | 400 µg $ml^{-1}$ |
| Immature tubers (6MAP) | 0.01 | 0.01 | 0.01 | 0.03 |
| Mature tubers (21MAP) | 0.03 | 0.03 | 0.09 | 0.12 |
| Over mature tubers (36MAP) | 0.03 | 0.07 | 0.08 | 0.12 |
| Standard ascorbate | 0.38 | 0.62 | 0.63 | 0.67 |

Reducing power of standard ascorbate and milk yam tubers irrespective of the maturity stages increased in a concentration dependent manner. At 400 µg $ml^{-1}$ concentration, absorbance recorded were 0.028, 0.118 and 0.119 nm by immature, optimally mature and over mature tubers respectively and the reference standard ascorbate recorded 0.672 nm.

### *In vitro* Anti-diabetic Activity (Inhibition per cent)

Alpha amylase inhibition per cent recorded for evaluating anti-diabetic activity of milk yam tuber extracts (petroleum ether, chloroform, ethanol and water) of different maturity stages were found significantly different between the different solvent extracts, maturity stages as well as their interactions and are tabulated in Table 4.

Among the different solvent extracts of milk yam tubers, maximum per cent of alpha amylase inhibition was recorded by aqueous extracts, 89.40 per cent followed by ethanolic extracts (87.53 per cent) and the least being recorded by petroleum ether extract (56.87 per cent).

**Table 4:** Alpha amylase inhibition activity of different solvent extracts of milk yam (*Ipomoea digitata* L.) tubers at different maturity stages, per cent

| | Inhibition percent of alpha amylase enzyme | | | | |
|---|---|---|---|---|---|
| | Petroleum ether (per cent) | Chloroform (per cent) | Ethanol (per cent) | Water (per cent) | Mean (per cent) |
| Immature tubers | 64.87 | 90.73 | 64.07 | 70.33 | 72.50 |
| Mature tubers | 45.43 | 52.73 | 99.20 | 98.33 | 73.93 |
| Over mature tubers | 60.30 | 95.60 | 99.33 | 99.53 | 88.70 |
| Mean (per cent) | 56.87 | 79.69 | 87.53 | 89.40 | |
| | S.Em± | | | CD (P=0.05) | |
| Solvent | 0.16 | | | 0.47 | |
| Maturity | 0.14 | | | 0.41 | |
| Solvent x Maturity | 0.28 | | | 0.81 | |

Over mature tubers recorded significantly highest per cent of alpha amylase inhibition, 88.70 per cent than optimally mature tubers (73.93 per cent) and the least being recorded by immature tubers, 72.50 per cent.

Interaction between maturity stages of milk yam tubers and the different extracts revealed dominant per cent of alpha amylase inhibition by aqueous extract of over mature milk yam tubers (99.53 per cent). Dominant activity was recorded by ethanolic extract of optimally mature (99.20 per cent) and over mature (99.33 per cent) milk yam tubers as well. Petroleum ether extract of mature milk yam tubers recorded significantly less inhibition, 45.43 per cent.

## Discussion

*In vitro* pharmacological properties viz., antioxidant and anti-diabetic activity of milk yam tubers at immature (six MAP), optimally mature (21 MAP) and over mature (24 MAP) stages were evaluated with respect to inhibition per cent which significantly differed among themselves and are discussed herewith.

### Antioxidant Activity of Milk Yam Tubers

DPPH free radical scavenging activity, nitric oxide radical scavenging activity and reducing power assay were carried out for authenticating the anti-oxidant potential of optimally mature milk yam tubers (21 MAP) by comparing it with immature and over mature ones and are discussed below.

It is clear from the Fig. 1, Fig. 2 and Fig. 3 that inhibition per cent of DPPH radicals and nitric oxide radicals as well as reducing power (nm) increased in a concentration dependent manner for the tuber samples and the reference standard (ascorbate for DPPH and reducing power assay) appraised. At

lower concentrations, DPPH radical inhibition activity of over mature milk yam tubers exceeded the reference standard ascorbate (Table 1.). Despite the concentration dependent enhancement in per cent inhibition, at a concentration of 600 µg $ml^{-1}$ or more, milk yam tubers had inferior activity than the reference standard ascorbate.

Moreover, among the tuber samples evaluated for DPPH radical scavenging activity, over mature tuber recorded predominant activity than others. Besides, optimally mature and over mature tubers registered comparable inhibition per cent of nitric oxide radicals in all the concentrations tried and were higher than the immature ones (Table 2.). Fig. 3 displayed considerably higher reducing power for standard ascorbate than milk yam tuber samples of all maturity. Similar pattern of increment in reducing power with increase in concentration was noted in *Curcuma longa*, *Tribulus terrestris*, *Bacopa monnieri* [19] and sweet potato leaf extracts [10]. Over and above this, reducing power of optimally mature milk yam tubers showed a steep increase from 100 to 200 µg $ml^{-1}$ and hence, reducing power of optimally mature and over mature tubers were identical at 200 µg $ml^{-1}$ or higher concentration which was higher than the immature tubers (Table 3.). Based on the results, milk yam tubers of 21 MAP could be considered as potent antioxidant source which is not in agreement with the $IC_{50}$ value (230 µg $ml^{-1}$ and 50 µg $ml^{-1}$), recorded for DPPH radical scavenging activity and FRAP reducing power activity respectively of milk yam tubers reported by Vasagam *et al*. [33] Such a surpassing discrepancy with data of present study might be ascribable to genetic factors [15], geographical variation, difference in harvesting season [6] etc.

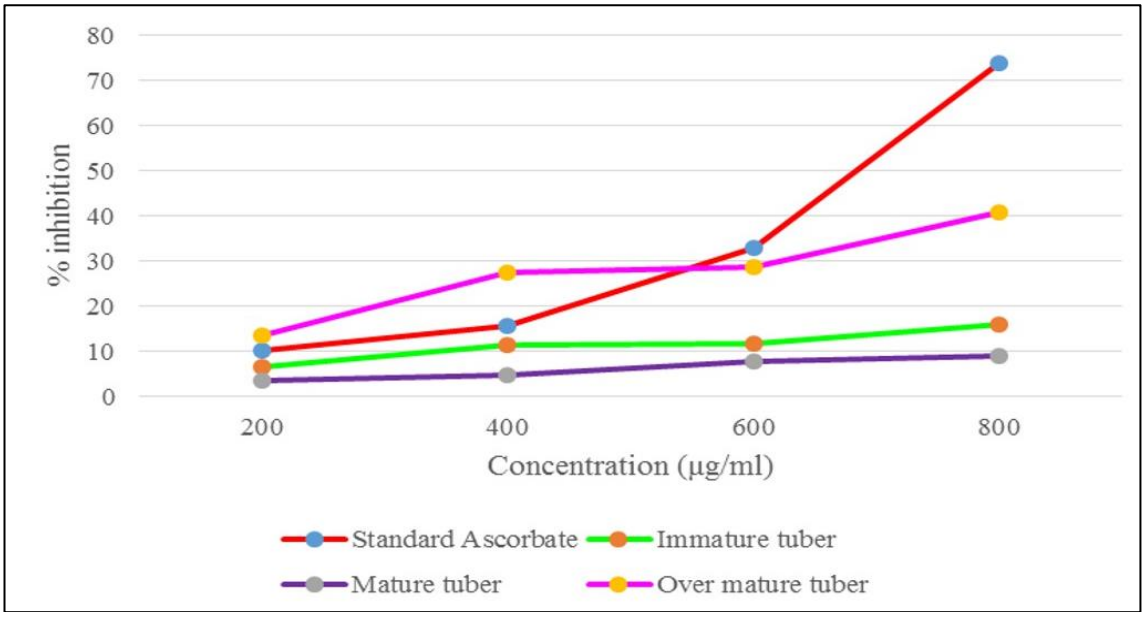


**Fig 1:** DPPH radical scavenging activity of standard ascorbate and milk yam (*Ipomoea digitata* L.) tubers at different maturity stages

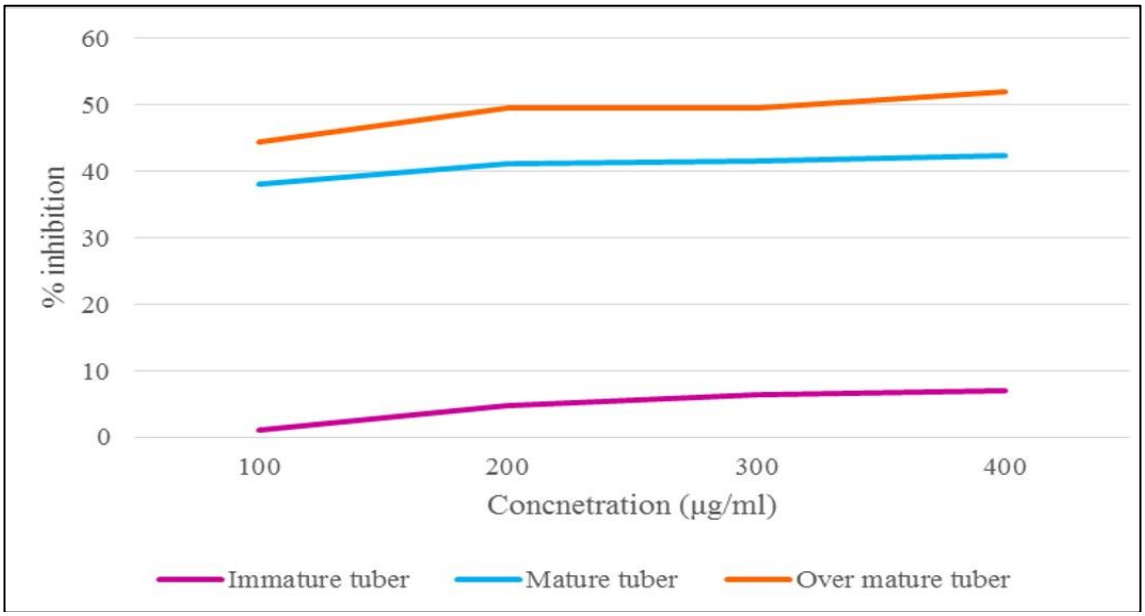


**Fig 2:** Nitric oxide radical scavenging activity of milk yam (*Ipomoea digitata* L.) tubers at different maturity stages

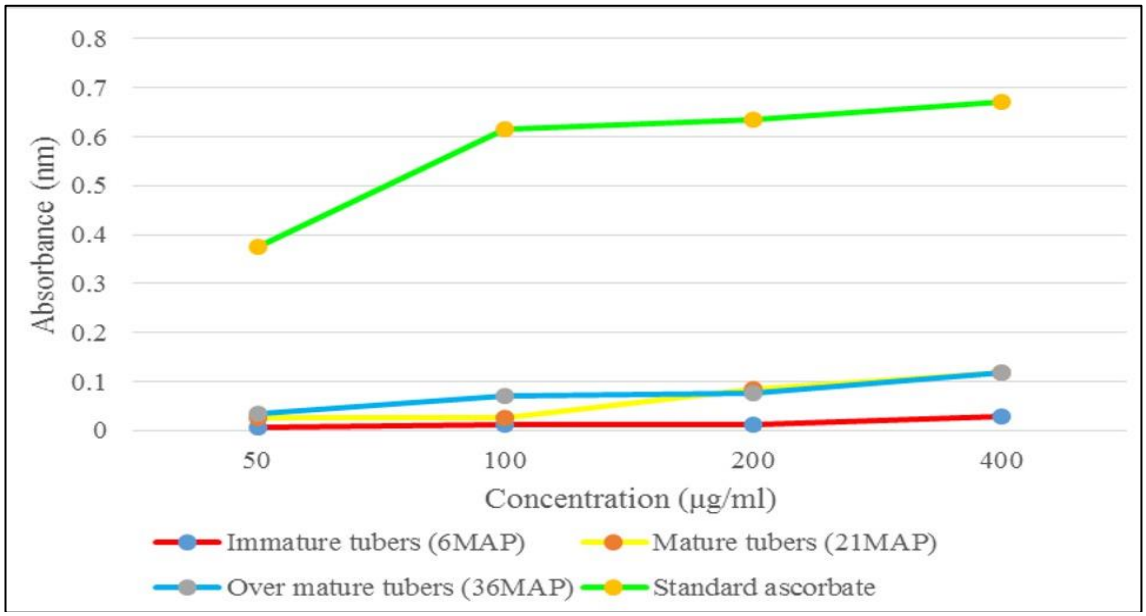


**Fig 3:** Reducing power of standard ascorbate and milk yam (*Ipomoea digitata* L.) tubers at different maturity stages

According to many reviews, antioxidant activity of a crude drug showed good relationship with phytochemicals particularly phenolic compounds such as flavonoids, phenolic acids, tocopherol etc. [8, 2, 4, 17] Phenolic compounds contained phenolic hydroxyl groups which effectively donated hydrogen ions or acted as oxygen quenchers and performed antioxidant activity[18]. In the present study, higher antioxidant activity was recorded in mature milk yam tubers which might be ascribed to higher flavonoid content (0.54 mg QE $g^{-1}$). One the other hand, milk yam tubers of six MAP that recorded similar flavonoid concentrations (0.57 mg QE $g^{-1}$) had lower antioxidant activity. This controversy needed to be clarified by exploring and extending the data by evaluating other phenolic compounds that could contribute higher antioxidant activity to optimally mature tubers. Moreover, components in milk yam tubers such as β-sitosterol [23], Umbelliferone [11], Rutin [34] etc. in milk yam tubers are assignable to higher antioxidant activity and can be considered as potent nutraceuticals [22].

**Anti-diabetic Activity of Milk Yam Tubers**

Diabetes is often referred to as a syndrome that requires lifelong treatment [9]. Report says, that about 346 million people are its sufferers and it may be doubled by 2030[20]. Currently, alpha amylase inhibitors attract considerable attention for its therapeutic potential in treatments of diabetes. Numerous botanical entities including milk yam tubers are reported to possess alpha amylase inhibition activity [30]. In the present work, alpha amylase inhibition activity of different solvent (petroleum ether, chloroform, ethanol and water) extracts of immature, optimally mature and over mature milk yam tubers were found significantly different and the results are discussed underneath.

Fig. 4. clearly appreciated that all the tuber extracts of milk yam tuber irrespective of its maturity stage exhibited alpha amylase inhibition activity. Aqueous and ethanolic extract of over mature and optimally mature milk yam tubers followed by aqueous extract of optimally mature tubers exhibited superior inhibition of alpha amylase enzyme (Table 4.). Among the milk yam tuber extracts, aqueous extract possessed dominant alpha amylase inhibition activity and was followed by ethanolic extract. Superior inhibition activity of aqueous milk yam extract over ethanolic extract was already disclosed [5, 13]. Petroleum ether extract of milk yam tubers recorded least inhibition and the same was documented by Chandira and Jayakar [5] as well. Yet, inhibition per cent was more than fifty (56.87 per cent). From Fig. 4 it could also be inferred that over mature tubers were endowed with augmented anti-diabetic activity (88.70 per cent) and was followed by optimally mature (73.93 per cent) and immature ones (72.50 per cent). Only few works are there in literature for comparing the alpha amylase inhibition activity of milk yam tubers at different maturity stages. Still, a positive relationship between antioxidant activity and anti-diabetic activity could be hypothecated in the present study also. Kumar *et al*. [15] had rationalized the relationship between anti-oxidant activity and anti-diabetic activity of *Aegle marmelos*. Moreover, they justified the puissance of the phytochemical component, Umbelliferone as an antioxidant and anti-diabetic agent. Accordingly, anti-diabetic action of Umbelliferone which was already authenticated in milk yam tubers through this research is not abstainable and its potentialities need to be explored further.

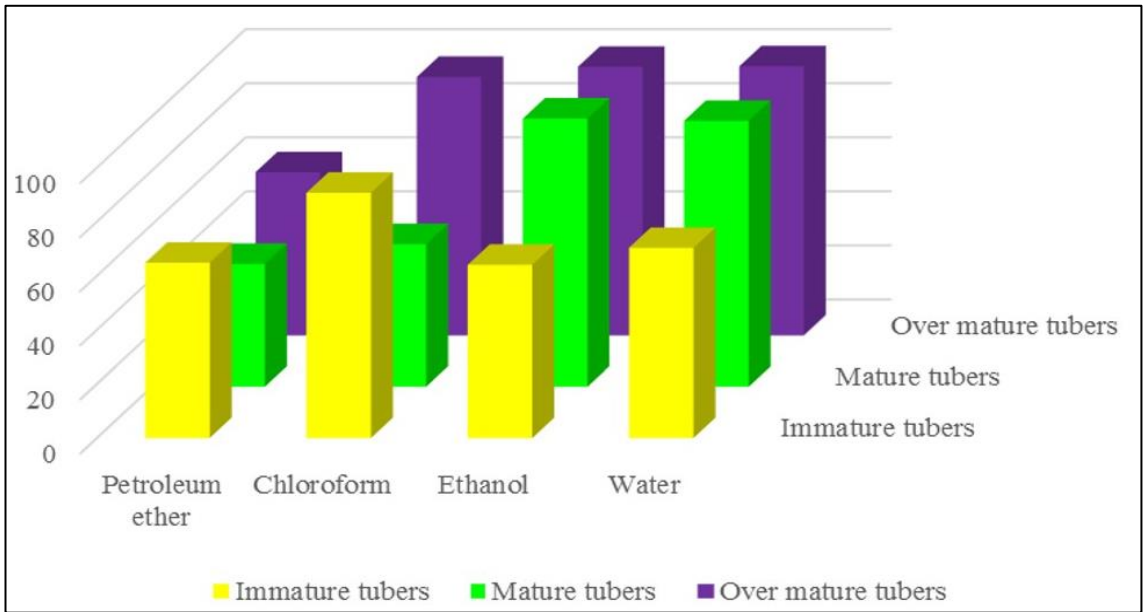


**Fig 4:** Alpha amylase inhibition activity of milk yam (*Ipomoea digitata* L.) tuber extracts

## Conclusion

Pharmacological properties viz., *in vitro* antioxidant activity (DPPH radical scavenging activity, nitric oxide radical scavenging activity and reducing power) and *in vitro* anti-diabetic activity of milk yam tubers were also found to vary with maturity. DPPH radical scavenging activity and nitric oxide radical scavenging activity of optimally mature milk yam tubers (21 MAP) recorded least $IC_{50}$ values, 1040.00 µg $ml^{-1}$ and 355.00 µg $ml^{-1}$ respectively. Reducing power of optimally mature milk yam tubers observed as absorbance at 400 µg $ml^{-1}$ concentration was 0.118 nm. Dominant *in vitro* anti-diabetic activity measured as alpha amylase inhibition activity of milk yam tubers were recorded by aqueous extracts, 89.40 per cent and over mature tubers, 88.70 per cent. Ethanolic extract of optimally mature tubers recorded superior *in vitro* anti-diabetic activity (99.20 per cent).

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