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Medicinal plants with antioxidant potential of leaf extracts of selected medicinal plants

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Abstract

Traditional system of medicine is a part of Indian life. The present study was carried out to know the anti oxidant activity of selected medicinal plants viz, *Aegle marmelos*, *Butea monosperma*, *Commifora wightii*, *Holostemma ada-kodien*, *Decalepis hamiltonii*, *Gloriosa superba*, *Gymnema sylvestre* and *Santalum album* were studied from Indalwai forest of Nizamabad. DPPH, Nitric Oxide, Superoxide, Hydrogen peroxide scavenging activity of methanol leaf extracts at different concentrations were studied (ascorbic acid used as standard). The present study revealed the anti oxidant activity of the leaf extracts of selected plants at all concentrations tested.

Keywords: Anti oxidant, *Aegle marmelos*, *Gloriosa superba*, *Gymnema sylvestre* and *Santalum album*, Ascorbic acid

Introduction

Ayurveda is the oldest and offers a holistic approach and it is popular in practice since the Vedic period. Medicinal plants are connecting links between the traditional (Homeopathic) and modern (Allopathy) systems. In India traditional system almost runs parallel to the modern medicine health care providers. Government of India officially recognized and established AYUSH ministry.

Since time immemorial, medicinal plants have been exploited for their medicinal use. Nature has been the source a large number curative compounds in addition to the resources. The use of ancient traditional medicine is a part of the people in the third world countries. There is an ever increasing demand for extraction, isolation, purification and development of drugs for chemotherapy from traditional medicinal plants. (UNESCO, 1998) [1].

The phytochemicals in medicinal plants are responsible for the activities like antioxidant, antimicrobial and antipyretic (Cowman, 1999; Adesokan *et al.*, 2008) [2, 3]. As per W.H.O, plants are life saving as they are sources of drugs and should be screened for their safety and efficacy (Nascimento *et al.*, 2000) [4]. Evidences suggest that humans might have employed the pharmacological properties of plants and their parts to treat the diseases since the inception of civilization. The phenolic and flavonoids are antioxidant and aids in free radical scavenging activity along with anti-inflammatory and anti cancerous in nature (Asha *et al.* 2011) [8].

Antioxidants scavenge free radicals, and convert reactive oxygen species, antioxidant nature is due to phytochemicals. As of late developing proof has been collected demonstrating the contribution of reactive oxygen species (ROS) in the pathogenesis of numerous sicknesses (Halliwell *et al.*, 1992). These ROS significantly targets lipids, proteins, catalysts, RNA and DNA, brought about unsettling influences of redox balance in a solid body and therefore causes degenerative/oxidative sicknesses, like cardiovascular infections, malignant growth, neuro-degenerative malady and so forth (Ames *et al.*, 1993) [6].

Deepa Babu *et al.*, (2013) [7] have assessed the antioxidant activity of the ethanolic extract of "Triphala". Polyphenols in Triphala exerts anti oxidant activity. The flavonoids, saponins and tannins may be dependable to some extent for the observed pain relieving and calming impact. The utilization of medicinal plants is wide spread internationally and it's increasing day by day and due to the advantages of traditional medicine and its outreach it was planned to study the anti oxidant nature of *Aegle marmelos*, *Butea monosperma*, *Commifora wightii*,

Holostemma ada-kodien, *Decalepis hamiltonii*, *Gloriosa superba*, *Gymnema sylvestre* and *Santalum album* leaf extracts.

Materials and Methods

DPPH radical scavenging activity

The total anti-oxidant potential was determined by Brand-Williams *et al.*, and Parejo *et al.*, Various concentrations of test sample were prepared by serial dilution and 0.1mL of each dilution was added to 3.9 mL of a 6.0×10^{-5} μ M methanol solution of DPPH, followed by vortexing. The reaction was allowed to take place in the dark at room temperature to reach a plateau.

The decrease in the absorbance was measured at 517 nm was determined by using a Shimadzu spectrophotometer. The concentration of remaining DPPH in the reaction medium was calculated from the calibration curve as follows:

$$\text{Scavenging effect (\%)} = \frac{(1 - A \text{ Sample (517nm)})}{A \text{ Control (517nm)}} \times 100$$

Super oxide free radical scavenging activity

Different concentrations of 50, 100, and 150 μ g/mL (10, 20, 30 μ L) of plant extracts were taken and the volume was made up to 150 μ L with methanol, to each of this, 100 μ L of riboflavin, 200 μ L EDTA, 200 μ L methanol and 100 μ L NBT was mixed in test tubes and further diluted up to 3 mL with phosphate buffer and absorbance was measured after illumination for 5 min, at 590 nm on UV visible spectrophotometer (Shimadzu, UV-1601), Japan and results were compared with ascorbic acid (10 μ g/mL as standard).

Scavenging of nitric oxide

Sodium nitroprusside (5 μ M) in standard phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025 M, pH 7.4) and the tubes were incubated at 25°C for 5 h. After 5 h, 0.5 mL of incubation solution was removed and diluted with 0.5 mL Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthyl ethylene di amine dihydro chloride in water). The absorbance of chromophore formed was recorded at 546 nm.

The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The activity was compared with ascorbic acid.

Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various

concentrations of 1mL of the extracts or standards in methanol were added to 2 mL of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without hydrogen peroxide. IC₅₀ value is the concentration of the sample required to scavenge 50% free radical.

The percentage inhibition was calculated by using the following formula.

$$\text{Scavenging activity (\%)} = \frac{\text{OD of control} - \text{OD sample}}{\text{OD of control}} \times 100$$

Results and Discussion

The present study, DPPH scavenging activity revealed the leaf extracts of *Aegle marmelos*, *Butea monosperma*, *Commifora wightii*, *Holostemma ada-kodien*, *Decalepis hamiltonii*, *Gloriosa superba*, *Gymnema sylvestre*, *Santalum album* possesses scavenging activity at the studied concentrations.

Pairing of unpaired electrons results in neutralization and converts it into 1-1 di phenyl-2- picryl hydrazine and becomes colorless from purple color.

The DPPH radical was measured at 517 nm. DPPH, Superoxide, Nitric oxide, Hydrogen Peroxide scavenging activity of *Aegle marmelos*, *Butea monosperma*, *Commifora wightii*, *Holostemma ada-kodien*, *Decalepis hamiltonii*, *Gloriosa superba*, *Gymnema sylvestre* and *Santalum album* methanol leaf extracts at different concentrations measured [ascorbic acid (10 μ g/ml) was used as standard]. The present study revealed that the *Aegle marmelos*, *Butea monosperma*, *Commifora wightii*, *Holostemma ada-kodien*, *Decalepis hamiltonii*, *Gloriosa superba*, *Gymnema sylvestre* and *Santalum album* methanol leaf extracts were observed for significant reduction of the super oxide anions and they inhibited the formation of formazan.

It interacts with O₂ and results in nitrites and peroxy nitrites formation. Sodium nitroprusside at an optimum pH results in nitric oxide formation and it reacts with O₂ and forms nitrate ions, they are quantitatively measured by use of Griess reagent. The leaf extracts of selected plants were potent scavengers of nitric oxide and subsequently competes with oxygen (to inhibit the production of nitric oxide). H₂O₂ oxidizes thiol (-SH) groups and inactivates some enzymes. The cytotoxicity of H₂O₂ is due to hydroxyl radical generated by H₂O₂ interaction with Fe⁺² and Cu⁺² in the cell. Methanol leaf extracts were noticed for significant inhibitory activity of H₂O₂ due to the antioxidant and free scavenging of radical activity (Table no's 1-8 and Figure no's 1-8).

Table 1: Anti oxidant activity of *Aegle marmelos* (All values in this table represent the mean \pm SD (n=6))

<i>Aegle Marmelos</i>	Extract 50 μ g/ml	Extract 100 μ g/ml	Extract 150 μ g/ml	Standard 10 μ g/ml
DPPH	68 \pm 0.17	75 \pm 0.18	82 \pm 0.59	92 \pm 0.05
Superoxide	38 \pm 0.19	43 \pm 0.84	52 \pm 0.71	68 \pm 0.11
Nitric Oxide	66 \pm 0.14	68 \pm 0.21	70 \pm 0.32	80 \pm 0.14
Hydrogen Peroxide	37 \pm 0.22	41 \pm 0.41	51 \pm 0.71	67 \pm 0.16

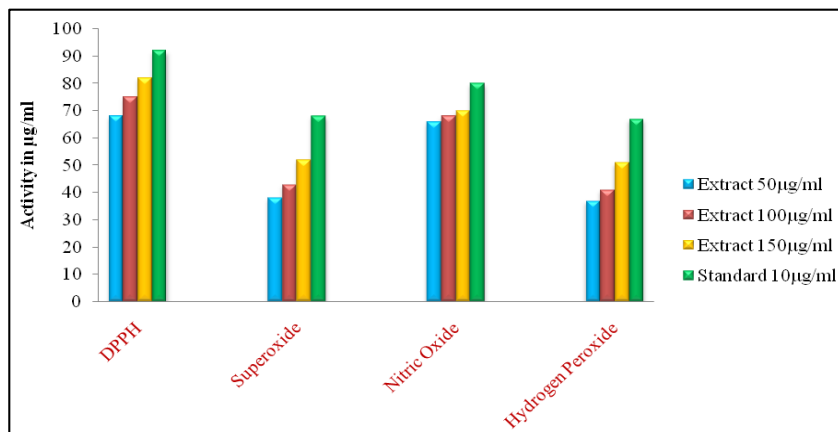


Fig 1: Anti oxidant activity of *Aegle marmelos*

Table 2: Anti oxidant activity of *Butea monosperma*. (All values in this table represent the mean ± SD (n=6))

<i>Butea monosperma</i>	Extract 50µg/ml	Extract 100µg/ml	Extract 150µg/ml	Standard 10µg/ml
DPPH	62 ±0.21	73 ±0.11	80 ±0.49	92 ±0.16
Superoxide	37± 0.14	41±0.33	50 ±0.48	68±0.19
Nitric Oxide	65 ±0.21	67±0.18	69 ±0.52	80±0.14
Hydrogen Peroxide	36± 0.31	41±0.19	50±0.61	67±0.13

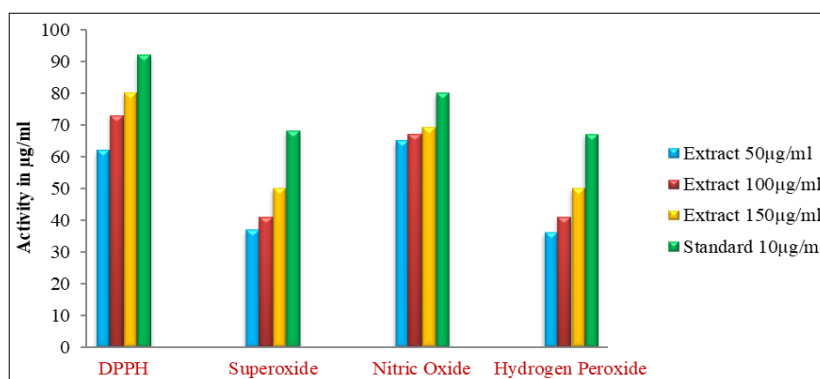


Fig 2: Anti oxidant activity of *Butea monosperma*

Table 3: Anti oxidant activity of *Commifora wightii* (All values in this table represent the mean ± SD (n=6))

<i>Commifora wightii</i>	Extract 50µg/ml	Extract 100µg/ml	Extract 150µg/ml	Standard 10µg/ml
DPPH	63 ±0.14	74 ±0.41	81 ±0.16	92± 0.11
Superoxide	38± 0.18	40±0.43	51±0.17	68±0.12
Nitric Oxide	66±0.19	67±0.49	70±0.12	80±0.13
Hydrogen Peroxide	37±0.41	42±0.47	51±0.13	67±0.16

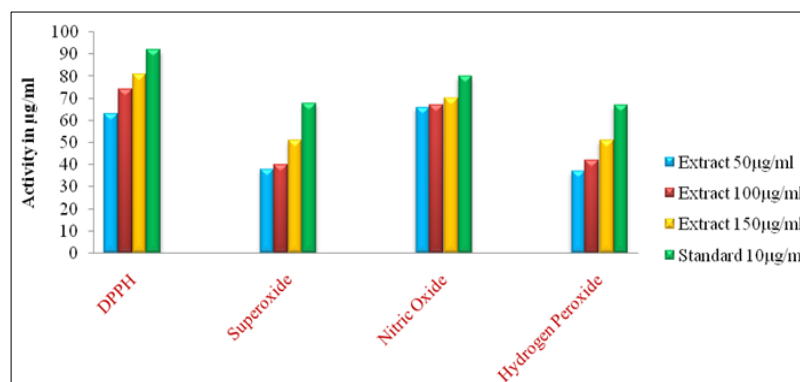


Fig 3: Anti oxidant activity of *Commifora wightii*

Table 4: Anti oxidant activity of *Holostemma ada-kodien* [All values in this table represent the mean ± SD (n=6)]

<i>Holostemma ada-kodien</i>	Extract 50µg/ml	Extract 100µg/ml	Extract 150µg/ml	Standard 10µg/ml
DPPH	60 ±0.17	70±0.39	80±0.41	92 ±0.04
Superoxide	40±0.16	40±0.33	50±0.44	68±0.12
Nitric Oxide	60±0.21	65±0.34	70±0.35	80±0.16
Hydrogen Peroxide	35±0.26	35±0.31	50±0.34	67±0.12

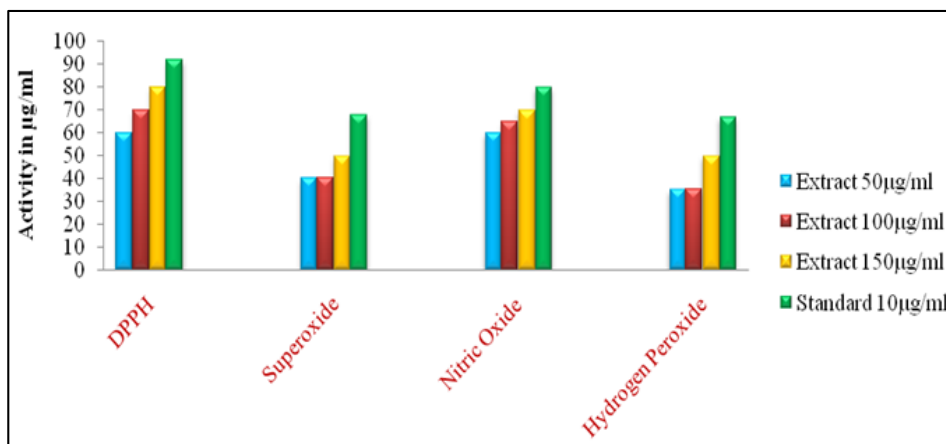


Fig 4: Anti oxidant activity of *Holostemma ada-kodien*

Table 5: Anti oxidant activity of *Decalepis hamiltonii* [All values in this table represent the mean ± SD (n=6)]

<i>Decalepis hamiltonii</i>	Extract 50µg/ml	Extract 100µg/ml	Extract 150µg/ml	Standard 10µg/ml
DPPH	65 ±0.21	75±0.31	85 ±0.17	92 ±0.12
Superoxide	44±0.28	44±0.33	55±0.54	68±0.18
Nitric Oxide	66±0.29	71±.38	75±0.24	80±0.10
Hydrogen Peroxide	38±0.24	40±0.23	58±0.26	67±0.13

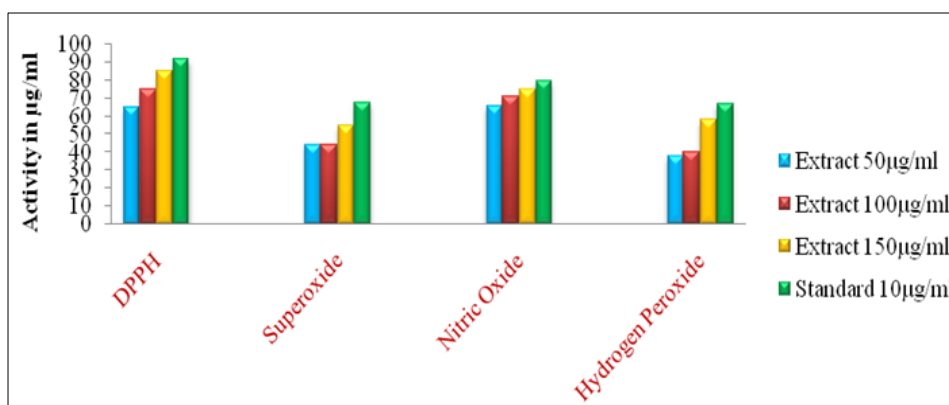


Fig 5: Anti oxidant activity of *Decalepis hamiltonii*

Table 6: Anti oxidant activity of *Gloriosa superba* (All values in this table represent the mean ± SD (n=6))

<i>Gloriosa superba</i>	Extract 50µg/ml	Extract 100µg/ml	Extract 150µg/ml	Standard 10µg/ml
DPPH	66±0.25	76 ±0.16	86±0.24	92±0.11
Superoxide	45±0.21	45±0.21	56±0.21	68±0.14
Nitric Oxide	67±0.22	72±0.18	76±0.24	80±0.16
Hydrogen Peroxide	39±0.19	41±0.17	59±0.19	67±0.10

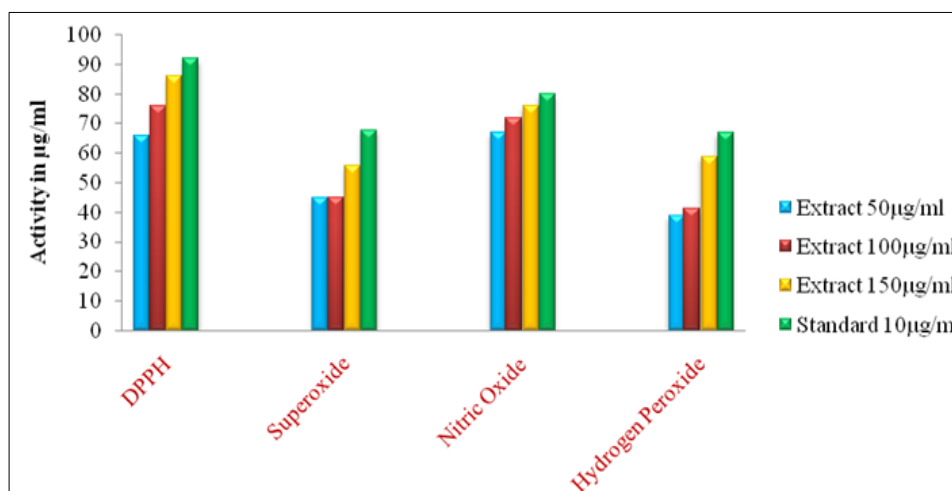
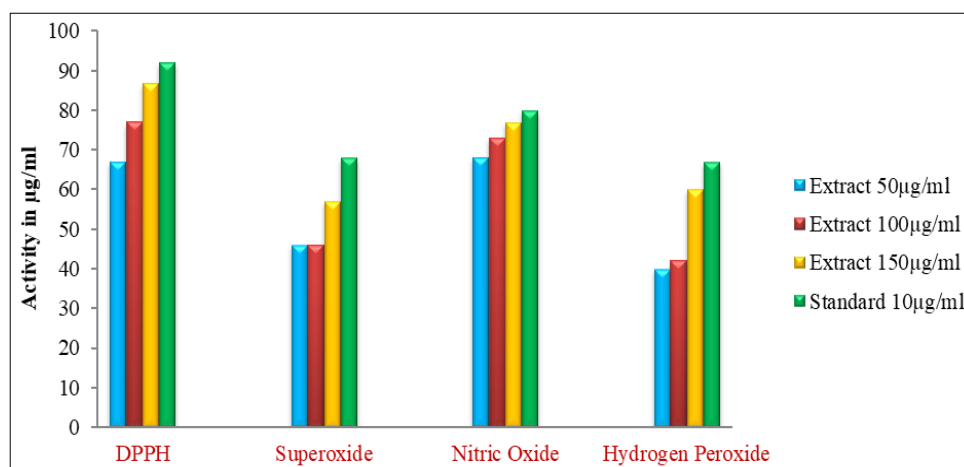


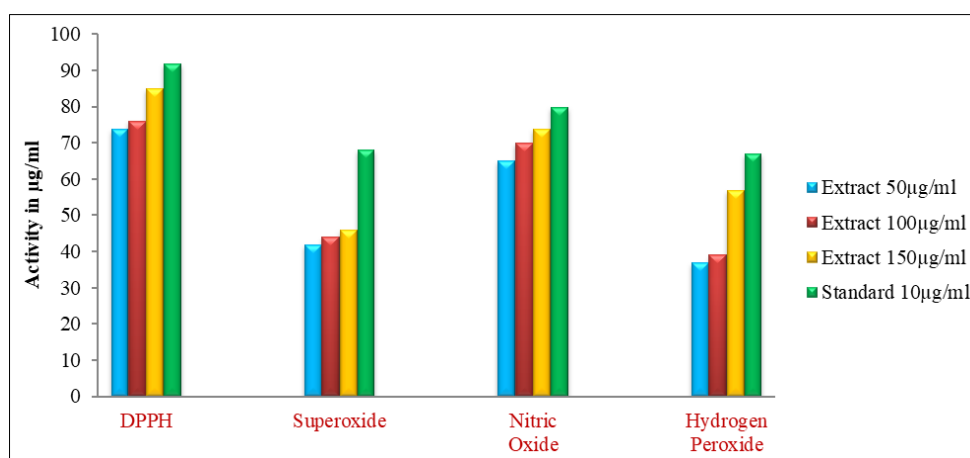
Fig 6: Anti oxidant activity of *Gloriosa superba*

Table 7: Anti oxidant activity of *Gymnema sylvestre* (All values in this table represent the mean \pm SD (n=6))

<i>Gymnema sylvestre</i>	Extract 50 μ g/ml	Extract 100 μ g/ml	Extract 150 μ g/ml	Standard 10 μ g/ml
DPPH	67 \pm 0.18	77 \pm 0.16	87 \pm 0.31	92 \pm 0.10
Superoxide	46 \pm 0.24	46 \pm .21	57 \pm 0.18	68 \pm 0.15
Nitric Oxide	68 \pm 0.31	73 \pm .24	77 \pm 0.17	80 \pm 0.11
Hydrogen Peroxide	40 \pm 0.25	42 \pm 0.22	60 \pm 0.21	67 \pm 0.16

**Fig 7:** Anti oxidant activity of *Gymnema sylvestre***Table 8:** Anti oxidant activity of *Santalum album* (All values in this table represent the mean \pm SD (n=6))

<i>Santalum album</i>	Extract 50 μ g/ml	Extract 100 μ g/ml	Extract 150 μ g/ml	Standard 10 μ g/ml
DPPH	74 \pm 0.11	76 \pm 0.25	85 \pm 0.25	92 \pm 0.09
Superoxide	42 \pm 0.21	44 \pm 26	46 \pm 0.27	68 \pm 0.11
Nitric Oxide	65 \pm 0.24	70 \pm 0.29	74 \pm 0.31	80 \pm 0.15
Hydrogen Peroxide	37 \pm 0.19	39 \pm 0.18	57 \pm 0.34	67 \pm 0.12

**Fig 8:** Anti oxidant activity of *Santalum album*

The DPPH scavenging activity of selected plant extracts reveals anti oxidant activity of the leaf extracts of *Aegle marmelos*, *Butea monosperma*, *Commifora wightii*, *Holostemma ada-kodien*, *Decalepis hamiltonii*, *Gloriosa superba*, *Gymnema sylvestre* *Santalum album* exhibited good scavenging activity at all tested concentrations (ascorbic acid of 10 μ g/mL was used as standard).

4. Conclusion

The plants leaf extracts may be considered as a drug candidate, but requires further confirmation and safety and efficacy must be taken into account and there is a need to study the role of each individual components activity. Identification and isolation of individual phytochemical

components in the extracts also has to be done for further investigations.

There is a need to popularize the cultivation of Medicinal plants with the necessary packages of practices. Further pharmacological investigations are necessary.

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