

Original Research Article

Antioxidant Activity of “Nityaprasa” - A Polyherbal Nutraceutical Drug

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ABSTRACT

Keywords

Nityaprasa,
Nutraceutical,
Antioxidant
Activity,
Polyherbal
formulation

“Nityaprasa” An Ayurvedic poly herbal drug formulation was evaluated for its antioxidant potency. This formulation comprises of four plants namely *viz.*, *Dregea volubilis* (Asclepiadaceae), *Tiliacora acuminata* (Combretaceae), *Solanum seafortianum* (Solanaceae) and *Aristolochia indica* (Aristolochiaceae). The selected taxa can be grown in home gardens and known for their ornamental beauty. The drug formulation was done according to the ayurvedic norms. Different extracts of the prepared drug was tested for its antioxidant activity. Quantitative analysis of antioxidants, Antioxidant assays like DPPH, ABTS assays were evaluated. These results proved that “Nityaprasa” can be used as a household Nutraceutical supplement along with the regular diet.

Introduction

The nutraceutical and botanical terms are often used by the lay press or for marketing purposes to describe health beneficial food, food supplements or herbs. However, there is no common definition of nutraceuticals or botanicals and moreover a lack of regulation that classifies this category. Botanicals were often used as a synonym for herbs or herbal products with medicinal potency (Wildmann, 2001). Nutraceuticals as well as botanicals can be legally bought in pharmacies, supermarkets or online shops and sold as part of a normal diet. They contain substances, which could be beneficial for health by preventing or treating one or more diseases. In that way, they respond to the desire today’s consumer for self-medication and prevention of

disease risks (Fattinger and Meier-Abt,2003). There are an increasing number of nutraceutical supplements emerging in a variety of distribution channels, especially in the internet-based sales. At the same time, safety concerns of the governmental authorities lag behind the marketing strategies of distributors. In other words, there are more and more supplements with scarce scientific evidence of their medicinal potency, no or little control of their composition, no control of their shelflife and no knowledge of side effects or supplement-drug interactions (Kalra, 2003).

Hence there is considerable variation in the identity of the various source plants of the individual drugs selected for use. In

this context, there is a great need for the household "dietary supplements," which are in fact being utilized as non-prescription drugs for every human. In this research we have focused on the Scientific evaluation of four selected important medicinal taxa that possess potential antioxidant activity for the preparation of Nutraceutical drug. The aim of the present Biochemical evaluation is to prepare a Household Nutraceutical medicine for the daily use along with food.

The botanical identification of the taxa was carried out by using regional and local floras (Gamble, 1957; Pullaiah *et al.*, 2001, Yasodamma and Binny, 2013 and Madhava Chetty *et al.*, 2015). The herbarium was prepared according to the method of Jain and Rao (1977) and deposited in the department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh for further use.

Preparation of Nityaprasa

Solanum seaforthianum Mature and immature fruits are crushed and made into paste (100gms) and the mixed with 100 gms of *Teliocora acuminata* Mature fruits pulp. To this leaf paste of *Aristolochia indica* was mixed. This triple mixture was made to fry in *Dregea volubilis* Fruit oil. The approximate ratio formulated is made according to the diet followed. This preparation was made into aqueous extract for Biological evaluation.

Extract preparation

Preparation of Extracts were done according to the methodology followed by Vaghasiya and Chanda, (2007). The polyherbal formulation was mixed according to the dosage in equal proportions and was air dried at room temperature under shade for 3 weeks and grinded to 60 mesh size by using Willy Mill. Powder of 100 g was

soaked in 200 mL of 95% methanol (3 times) and filtered the extract with Whatman No. 1 filter paper. Filtrate was dried under vacuum by using rotary evaporator. The extract was suspended in distilled water and residual was used as aqueous extract. Extract was dried by using rotary evaporator and preserved at 40 °C (Kim DO, 2003).

Quantitative analysis of antioxidants

Quantitative analysis of antioxidants were done according to the methodology followed by Mitta *et al* (2015) Quantification of Total Proanthocyanidins (TPA) were quantified according to standard calorimetric method reported by Sun *et al.*, (1998) and total content of proanthocyanidins was expressed in terms of catechin equivalent, CAE, mg of CA/g of dry extract. Quantification of Total Flavonoid Content (TFC) was followed according standard colorimetric assay (Yong SP, 2008). Rutin was used for plotting calibration curve. Total content of phenols was expressed in terms of rutin equivalent, RE, mg of R/g of dry extract.

Quantification of Total Flavonols (TF) were quantified according to the protocol followed by Kumaran and Karunakaran (2007) with slight modifications. Total content of flavonols was expressed in terms of quercetin equivalent, QUE, mg of QU/g of dry extract. Quantification of Total Phenolic Content (TPC) Folin–Ciocalteu reagent were analyzed as per Kim *et al.*, (2003) methodology. Total content of phenols was expressed in terms of gallic acid equivalent, GAE, mg of GA/g of dry extract.

Antioxidant assays

Scavenging activity of DPPH radicals

The scavenging activity of DPPH was assessed by scavenging of 2, 2-diphenyl-1-

picrylhydrazyl radicals by employing the method of Brand-Williams et al., (1995). 2.4 mg DPPH was dissolved in 100 ml of methanol to prepare the stock solution of DPPH. The DPPH solution was diluted with methanol to achieve an absorbance of 0.980 ± 0.02 at 517 nm. DPPH solution of 500 μ l was added to 500 μ l of the dried fractions at varying concentrations (25–250 μ g/ml) and vibrated vigorously. After the incubation of 15 min in the dark, absorbance was recorded at 517 nm. The DPPH scavenging activity of various fractions was calculated by the following equation:

Percentage inhibition (%) =

$$\frac{[(\text{Absorbance of control} - \text{Absorbance of sample}) / (\text{Absorbance of control})] \times 100}{}$$

IC₅₀ values obtained as to determine the 50% inhibition of DPPH radicals. Ascorbic acid has used as standard.

Total antioxidant capacity (TAC)

Reduction of phosphomolybdenum was calculated to determine the total antioxidant capacity by adapting the method of Umamaheswari and Chatterjee (2008). 100 μ l of extract was mixed with 1.0 ml of the reagent solution; consisting of phosphate buffer, 0.6 M H₂SO₄, 28 mM sodium molybdate and 4 mM ammonium molybdate. The mixture was incubated in a water bath at 95°C for 90 min. The absorbance was measured at 765 nm, after cooling the mixture at room temperature. A standard of ascorbic acid was employed. Total antioxidant scavenging capacity was calculated as:

Total antioxidant capacity (%) =

$$\frac{[(\text{Absorbance of control} - \text{Absorbance of sample}) / (\text{Absorbance of control})] \times 100}{}$$

IC₅₀ values obtained as to determine the Total antioxidant capacity. Ascorbic acid has used as standard.

Scavenging activity of ABTS (Azino-bis (3-ethylbanzthiazoline-6-sulphonic acid) radicals

ABTS scavenging activity was evaluated by following the standard Protocol followed by Re R *et al.*, (1999). To prepare a dark colored ABTS working solution, ABTS solution (7 mM) was mixed with potassium oxidopersulphate (2.45 mM) solution and was placed in the dark for 12–16 h. The solution was diluted with 50% methanol and absorbance was adjusted at 0.7 (± 0.02) at 734 nm. Extract (100 μ l) was mixed with 1 ml of ABTS working solution and decrease in absorbance was read 1 min after adding the extract and then up to 6 min. Percentage inhibition was calculated according to following formula:

$$\text{Inhibition (\%)} = \frac{[(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100}{}$$

IC₅₀ values obtained as to determine the 50% inhibition of ABTS radicals. Ascorbic acid has used as standard.

Statistical analysis

The entire work was carried in triplicates. Data was calculated and the graphs were plotted using the software Origin 7. (Software Inc., San Diego, CA, USA). Samples data was compared to their respective standards using paired t-test. The correlation between quantities of antioxidants and the antioxidative properties was done using data analysis tool pack in excel 2007. Results were considered statistically significant at P < 0.05.

Results and Discussion

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) Extraction of the Extracts

The TPC values ranged from 14.53 ± 2.66 mg of Gallic acid equivalents per g of dry weight to 99.13 ± 0.70 mg Gallic acid equivalents per g of dry weight. The TFC values ranged from 95.50 ± 3.14 mg of Catechin equivalents per g of dry weight to 305.51 ± 3.63 mg of Catechin equivalents per g of dry weight. The order of the different extracts based on their TPC and TFC values is: n-Hexane < Ethyl Acetate < Methanol (Table 1).

In-vitro antioxidant assays

The percentage of DPPH and Superoxide scavenging activity was increased with increase in the concentration of the extract (Graph 1, 2). The highest DPPH scavenging activity was exerted by aqueous extract following methanolic and ethyl acetate having the IC_{50} values 54.99 ± 3.76 , 168.21 ± 6.95 and 302.12 ± 3.0 respectively. The higher Superoxide scavenging capacity also exerted by aqueous extract following methanolic and ethyl acetate having the IC_{50} values 61.33 ± 3.25 , 225.68 ± 5.12 and 255.42 ± 20.45 respectively. All the extracts exerted noticeable TAC. It also increased with the increase in the concentration (Graph 3). The highest TAC exerted by aqueous extract following methanolic and ethyl acetate and their IC_{50} values are 60.89 ± 3.33 , 97.31 ± 4.05 and 515.09 ± 5.99 respectively. All the IC_{50} values were compared to its standard ascorbic acid. The antioxidant efficacy of standard ascorbic acid increased with increase in the concentration. The IC_{50} values of ascorbic acid for DPPH, Superoxide and TAC are 21.64 ± 0.27 , 24.33 ± 0.41 and 29.86 ± 0.40 respectively.

Correlations between *in-vitro* antioxidant assays and phenolic and flavonoid contents of the extracts

IC_{50} values of DPPH, Superoxide and TAC scavenging capacity correlated to TPC and TFC of the extracts (Table 2). A positive correlation was found for TPC and radical scavenging assays with significant R^2 values (0.781, 0.822) for IC_{50} values of DPPH, TAC and a weak positive correlation was found (0.477) for IC_{50} values of Superoxide scavenging activity of Ethyl acetate extract. Methanol extracts given a strong positive correlation for TPC and radical scavenging assays with significant R^2 values (0.999, 0.948, 0.969) for its IC_{50} values of DPPH, Superoxide and TAC. Aqueous extract had a strong positive correlation with TPC and radical scavenging assays with significant R^2 values (0.994) for IC_{50} values of DPPH, where as IC_{50} values of Superoxide and TAC had a positive correlation with TPC (0.735, 0.881).

A strong positive correlation was found for TFC and radical scavenging assays with significant R^2 values (0.960, 0.993, 0.939) for IC_{50} values of DPPH, Superoxide and TAC of Ethyl Acetate extract. Methanol extracts had a strong positive correlation with significant R^2 values (0.983, 0.988, 0.997) for IC_{50} values of DPPH, Superoxide and TAC and TFC. Aqueous extract had a strong positive correlation (0.999) for IC_{50} values of DPPH, where as the other antioxidant assays viz., Superoxide and TAC had a positive correlation with TFC (0.661, 0.827).

The results of DPPH scavenging activity had increased along with increase in the concentration. The IC_{50} values indicated the anti-radical capacity of the extracts. The aqueous extracts had the highest DPPH scavenging capacity whereas ethyl acetate extracts exerted the lowest capacity. The

present work showed the scavenging of the free superoxide radical by the application of the extract. The effect was concentration dependent and the solvent dependent. The Aqueous extract found to possess high

superoxide radical scavenging capacity following methanolic and ethyl acetate extracts. IC₅₀ values also indicated its superoxide radical scavenging capacity. All the extracts represented noticeable TAC.

Table.1 Plant materials

S.No	Plant taxa	Vernacular Names /Family	Part used
1	<i>Dregea volubilis</i> (Linn.) Benth. Asclepiadaceae	Tel: Kalisaku, Palagurija teega, Tummudu teega, Kodipala, Kurincha, Pedda-gurija, Bandi-gurija, Doodi-pala, Pala teega. Eng: Green wax flower, Sneezing silk cotton, Cotton milk plant.	Fruit oil
2	<i>Solanum seforthianum</i> Andrews Solanaceae	Eng: Potato creeper	Mature and immature fruits
3	<i>Teliocora acuminata</i> Miers. Menispermaceae	Teega muchidi, Kappa teega, Tivva muchidi, Nallangi teega, Verri chitramulamu, Nalla teega.	Mature fruits
4	<i>Aristolochia indica</i> L. Aristolochiaceae	Govela teega, Nalla eswari, Eswari, Irvaraveri, Dhula govela, Ettakalabandha. Eng: Indian birth-wort, Serpent root plant, Eswaran's root.	Leaves

Table.1 Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and Extraction Yield (EY) of the Extracts

Plant Extract	TPC (mg of gallic acid equivalents per g of dry weight)	TFC (mg of Catechin equivalents per g of dry weight)
Ethyl Acetate	14.53±2.66	95.50±3.14
Methanol	66.70±2.35	247.20±9.29
Aqueous	99.13±0.70	305.51±3.63

Values are the means of 'n' replicates±SD (n=3).

Table.2 Correlation between IC₅₀ values and TPC, TFC of Plant Extracts

Anti-oxidant property	Ethyl Acetate		Methanolic		Aqueous	
	TPC	TFC	TPC	TFC	TPC	TFC
DPPH	0.781**	0.960***	0.999***	0.983***	0.994***	0.999***
Superoxide	0.477**	0.993***	0.948***	0.988**	0.735***	0.661**
TAC	0.822**	0.939***	0.969**	0.997***	0.881***	0.827***

* Represents significant variation levels. *p≤0.05, **p≤0.01, ***p≤0.001, NS- not significant.

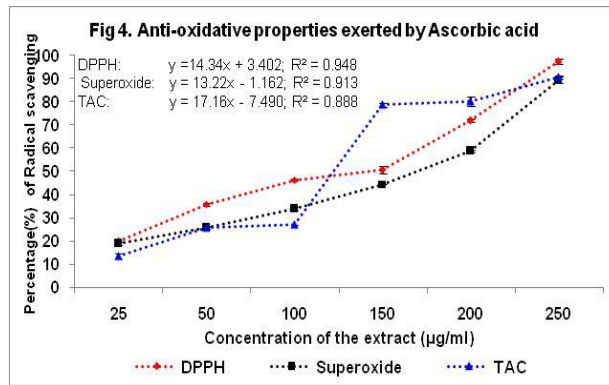
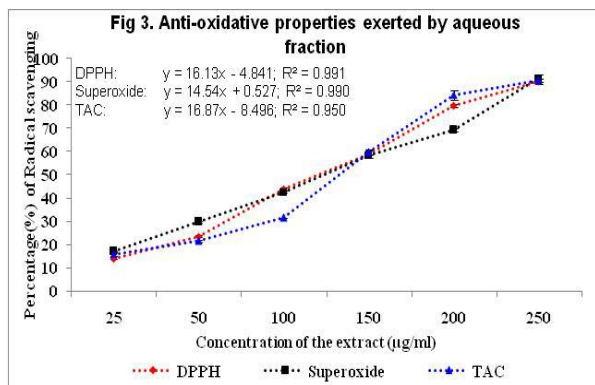
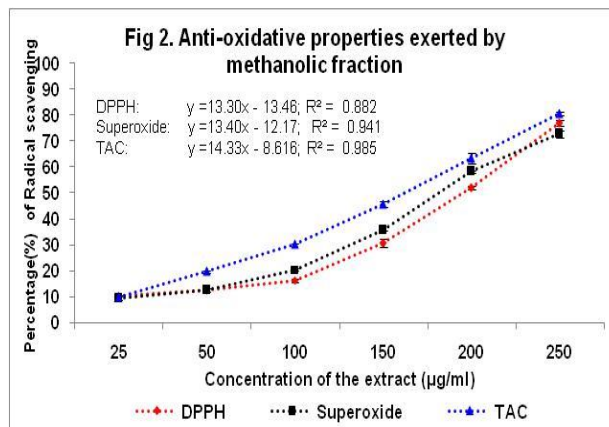
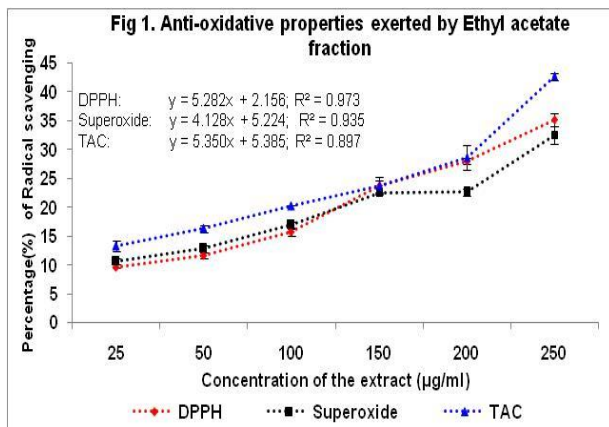
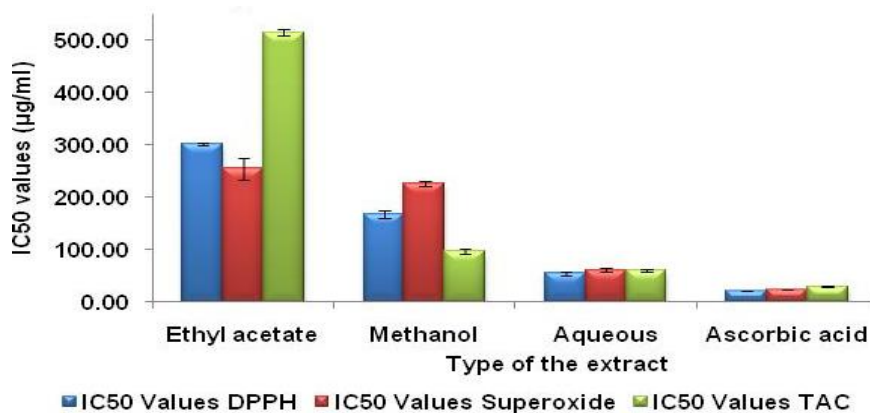


Fig.5 Comparison of Activity of different extracts with Standard



Based on the results of the DPPH, superoxide, TAC and ABTS, it was found that the extracts are natural antioxidants, it possess free radical scavenging efficacy. But the activity depends on the solvent and the concentration of the extract. The reason for

this antioxidative efficacy is due to their phenolic and flavonoid contents. Results showed that, total phenolic contents were solvent dependent. Highest TPC was found in aqueous whereas Ethyl acetate extract reflected small quantities of TPC. The TFC

values varied among all three of the extracts and the showed the order as in TPC i.e. EthylAcetate < Methanol < Aqueous.

The correlation results between TPC, TFC and Antioxidant assays strongly correlates the antioxidative properties of the extracts is as their phenolic and flavonoid contents. The antioxidant capacity was higher in the aqueous extracts following the methanolic and Ethyl acetate. The reason of high antioxidant activity of aqueous extract than other solvent extracts could be its phenolic and flavonoid contents as the aqueous extract showed high amount of TPC and TFC than other solvents. The order of the anti-oxidative capacity of the extracts is Hexane < Ethyl Acetate < Methanol.

Plants in this drug preparation have been widely studied and proved their Clinical studies which claimed for their Biological potency, pharmacological activities. Moreover, these selected taxa can be widely propagated in households and gardens. All the taxa are known for their ornamental beauty also.

In conclusion, the assays results indicated that it possess an antioxidant efficacy and can eliminate free radicals. The results also indicated that the antioxidant capacity is solvent and concentration dependent. The aqueous extracts found to be more effective among all. There was a positive correlation in between phenolic, flavonoid and radical scavenging assays, which strongly explains the reason of antioxidant capacity is as its phenolic and flavonoid contents. With this work, it can be concluded that the extracts are antioxidative in nature. The plant is a natural source of phenolics and flavonoids, which acts as anti-oxidants and can be used as a dietary food supplement in common household.

Acknowledgements

We would like to thank the Department of Botany, Sri Venkateswara University, Tirupati, India for providing all the facilities and support used to carry out the work.

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