Annals of
Plant Sciences
Volume 9, Issue 8 (2020) pp. 3970-3975
ISSN: 2287-688X
Research Article

Evaluation of Radical Scavenging Activity of Ethnomedicinal Syrup "*Nitya Yevvana Kashayam*"

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Abstract: This study aimed to evaluate the antioxidant compounds and their free radical scavenging capacity from Crude aqueous extract of ethnomedicated syrup "*Nitya Yevvana Kashayam* (NYK)". Estimation of antioxidant activity compounds *viz*., Total Phenolic Content (TPC), Total Flavonoids (TF) and Flavonol content (T.fl) were optimized by calorimetric methods respectively. DPPH and ABTS scavenging assay were used for the determination of free radical scavenging potentiality. Results reported that TPC (272.30 ± 0.44 mg GAE/gm DW), TF (158.65 ±0.82 mg QCE/gm DW) and the radical scavenging activity measurement, done with DPPH assay (EC_{50} = 22.24 ± 0.18 µg/mL) and ABTS assay (EC_{50} = 18.64 ± 1.35 µg/mL), present in NYK proved to be a good source of natural antiradical scavenger and therefore, recommended for utilization as potent medicine supporting the traditional ethnic claim.

Keywords: *Nitya Yevvana Kashayam; Ethnomedicinal syrup; Antioxidants; Talakona.*

Introduction

Natural antioxidants are widely considered effective antidote which reduce oxidative damage and exert beneficial effect for human health. Mining for medicated phytochemicals such as Flavonoids, Phenols which act as nutraceutical and antioxidant nature are since ancient times (Manuela and Huang 2018; Leite *et al*., 2018; Oliveira-Neto *et al*., 2017). Ethnobotanical surveys by various scientific folks done in Rayalaseema region of Andhra pradesh, India mentions that some of the forest associated Herbal healers (Village natu vaidyas, Tribal physicians) formulating and prescribing Nutraceutical and body relief giving ethnic plant drugs to their communities (Nagapadmavathi *et al*., 2020; Radhaiah *et al*., 2019; Savithramma *et al*.,2016; Vedavathy *et al*., 1997; Ganesh and Sudarsanam, 2013; Rao *et al*.,2006;). Our ethnobotanical explorations (Sripriya, 2017 a & b; Sripriya and Naik, 2019 a & b) in seshachalam hills and in Andhra pradesh reported huge ethnic claims on ethnic drugs for diverse ailments.

In this research, as a part of exploration on documentation of phytodrugs and after comprehensive perusal of traditional literature, documents and mining of tribal prescriptions, (Hemadri *et al*.,1987 a&b; Madhava chetty *et al*., 2018) we came across an interesting formulation, "*Nitya Yevvana Kashayam*"- an ethnomedicinal syrup prescribed by Tribal healers from Talakona forest habitats in Chittoor Dt. of Rayalaseema region of Andhra Pradesh. This formulation is prescribed as Neutraceutical, energy and immune boosting, discomfort and digestive stress related allied drug in the form of aqueous extract which was preceded since the generation. The plant species used to prepare NYK has been documented in Table 1. Figure 1.(A-E) represents the plant photographs used in NYK for easy identification.

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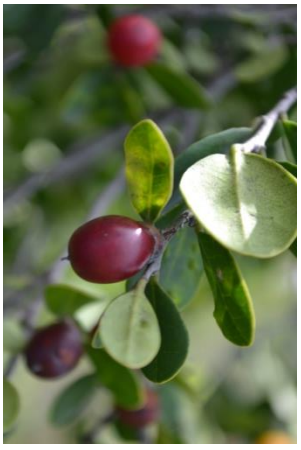

Figure **A**. *Phoenix loureiroi* **B.** *Maba buxifolia* **C.** *Gardenia gummifera* **D.** *Memecylon umbellatum*


E. *Phyllanthus emblica* **F**. *Ethnic healers collecting plant material for preparation of NYK*

Table 1: Detail information for the contents used for the preparation of NYK.

S.No	Name of the Plant	Family	Part used	Preparation/ Dosage
1.	*Phoenix loureiroi* Kunth	(Arecaceae)	Mature fruit (Mesocarp + Exocarp)	1kg fresh mature fruits collected and after removing seed squeezed and soaked in one litre water
2.	*Maba buxifolia* Pers	Ebenaceae	Mature ripen fruit	¼ kg of fruit pulp squeezed and a separate decoction prepared.
3.	*Gardenia gummifera* L.f.	Rubiaceae	Mesocarp	Outer later is removed and the mesocarp is used
4.	*Phyllanthus emblica* L.	Phyllanthaceae	Fruit without rind	200 mg of fruit pulp soaked in hot water for 2-3 Hrs.

Materials and Methods

Nitya Yevvana Kashayam is formulated in a dark closed room. Ingredients comprises of four plants with fruits *viz., Phoenix loureiroi, Maba buxifolia, Gardenia gummifera, Phyllanthus emblica* with some admixture for shelf life and easy intake. (Table 1) (Fig. A-F)

NYK Syrup Preparation : The raw material selected (Table 1) will be taken in a 5 Litre Pot and to that 3 L water should be added and ignited to make it in decoction form. To this little amount of Jaggery and ginger will be added for shelf life and for the sweetness. After

1 hr through mixing of all the material liquid will be extracted to a vessel and kept for 2-3 days. Admixtures can be taken with pepper, zinger, coriander, mentha leaf, pepper in warm water can be additionally added according as a flavor while in taking orally and should be taken half a glass (preferably clay vessel) as prescribed early morning before breakfast. Storage is not preferred since fresh syrup may get contaminated and poisoned. So, under the instructor surveillance only this ethnic medicine should be taken.

Interviews from the Tribal healers were taken according to the standard methodology adopted by Jain (1981) and herbarium was prepared according to Jain and Rao (1977).

Evaluation of Antioxidant compounds

Total phenolic content (TPC) determination

Total phenolic content (TPC) was analyzed using the Folin–Ciocalteu colorimetric method as suggested by Iqbal *et al*., (2015) and MahendraNath *et al.,* (2014) with some modifications. 1 mg of aqueous extracted NYK was dissolved in 10 ml of water and then mixed with 0.2 N Folin–Ciocalteu phenol reagent. After 5-6 min, 6% Na_2Co_3 (2.25 mL) was added and the mixture was incubated at 34 - 40° for one hour. The absorbance of the mixture was recorded at 760 nm with a spectrophotometer (Thermo scientific, USA). The results were recorded as the mean of three repetitive procedures and TPC is expressed as mg gallic acid equivalent (mg GAE) per gram of extract.

Total flavonoid content (TFC) determination

Total flavonoid content was determined using aluminum chloride ($Alcl_3$) colorimetric method (Chang *et al.,* 2002; Nath *et al*., 2013) with some modifications using quercetin as the standard. Briefly, 1 ml of 2 % $Alcl_3$ in water was mixed with an equal part volume of NYK aqueous extracted solution. The absorbance was taken at 435 nm for 20 min. The concentration of flavonoid (TFC) was expressed as mg quercetin equivalent (mg QE) per gram of extract.

Total flavonol content (TFl.C)

Total flavonol content was analyzed using the $AlCl_3$ colorimetric method (Al-Mamary *et al*., 2014; Radhaiah *et al*., (2019) with some modifications. Briefly, 1 mL of aqueous extract in different concentrations and then 2% $Alcl_3$ (1 mL), 5% sodium acetate (CH_3COONa) (3 mL) were added and assorted uniformly. At 3000 rpm, this mixture was then centrifuged for 20 min to get a clear solution. The absorbance of standard and sample were taken at 440 nm. Results were expressed as mg quercetin equivalent (QE) per gram of extract.

Radical scavenging activity-DPPH Assay (EC_{50})

The free radical scavenging capacity of aqueous extract of NYK extract was determined by using DPPH assay (Wang *et al.,* 2008; Olugbami *et al.,* 2015) with some modifications. Briefly, stock solution of the aqueous extract of NYK are diluted to final concentrations (from 0-3 mg/mL). 2 mL of DPPH solution at 80 mM, prepared in ethanol, is added to 0.1 mL of the extract solution. This mixture is then incubated at room temperature. After 30 min, the absorbance is measured at 517 nm using a spectrophotometer and then converted to percent inhibition of the DPPH radical. The % inhibition of both standard and samples was calculated.

Radical scavenging activity - ABTS assay (EC_{50})

Radical cation scavenging capacity of samples was examined against (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation (ABTS*+) described by Dong *et al.,* (2015) with some modifications (Radhaiah *et al.,* (2019). Briefly, 2 mL of the ABTS*+ solution was added to 100 µL of test samples at required concentration. The samples were mixed thoroughly, and the reaction mixtures were incubated at 34°C for 10 min, and the absorbance was recorded instantly at 734 nm. The % inhibition of both standard and samples were calculated and the concentration of ABTS content in the extract was reported as mg of trolox equivalent (TE)/g extract.

Both the assay's were carried out in triplicate. The % inhibition for DPPH and ABTS assay will be calculated according to the formula

$$\% \text{ radical scavenging activity} = (A_{control} - A_{sample}/A_{control}) \times 100$$

where A_{sample} is the absorbance of sample and $A_{control}$ is the absorbance of positive control (BHT).

Results and Discussion

Quantitative determination of antioxidants

The quantitative assessment for antioxidant compounds *viz.*, Total phenolic content (TPC), flavonoid content (FC), Total flavonols, Condensed Tanins (CT) in *Nitya yevvanna kashayam*, was analyzed with calorimetric assay and the results reported (triplicate - mean ± standard deviation) (Table 2).

Table 2. Quantitative determination of Antioxidant compounds

Variable	Quantity
TPC (mg GAE/gDW)	272.30 ± 0.44
TFC (mg QE/gDW)	158.65 ±0.82
Tfl (mg QE/gDW)	124.18 ± 0.72
TC (mg RU/gDW)	84.86 ± 2.46

RU = Rutin, GAE = Gallic acid equivalent, QE = Quercetin. The data represent the mean ± SD of triplicate assay for each sample.

The total phenolic content (TPC) of the extract was 272.30 ± 0.44 mg gallic acid equivalent/g dry weight. The total flavonoid content (TFC) of the extract was 158.65 ±0.82 mg of quercetin equivalent/g dry weight. The total flavanols 124.18 ± 0.72. The tannin content (CT) was 28.86 ± 2.46 rutin equivalent/g dry weight. The ascorbic acid content of the extract was 84.82 mg/g dry weight of extract.

Plant phenolic, flavonoid and flavonol compounds are the significant group of metabolites acting as free radical scavenging or primary antioxidants and therefore, it is justifiable to determine phenolic content (where flavonoids and flavanols are derived compounds) in aqueous crude extract. The presence of antioxidant compounds (TPC, TFC, T.fl) suggest that NYK is a rich source of natural antioxidants.

DPPH and ABTS assay (EC_{50} Value)

DPPH and ABTS assay measures Radical scavenging potency (Interms EC_{50} Value) of in the sample which are very simple, inexpensive and usually employed methods for the determination of antioxidant activity and can give good results (Iqbal *et al.,* 2015; Radhaiah *et al.*,2019). In this investigation, free radical scavenging ability of aqueous extract of NYK was estimated and the results were compared with BHT. (Table 3)

Table 3. Radical scavenging activity of aqueous extract of NYK and standards.

Assay /Control	EC_{50} Value
DPPH assay	22.24 ± 0.18 µg/mL
ABTS assay	18.64 ± 1.35 µg/mL
BHT (Control)	19µg/ mL

The results show that DPPH value (EC_{50}) as 22.24 ± 0.18 µg/mL) and ABTS assay (EC_{50} = 18.64 ± 1.35 µg/mL showed bit higher scavenging ability on DPPH and ABTS radicals when compared to the synthetic antioxidant BHT (Butylated hydroxytoluene, a water soluble analog) (EC_{50}= 19µg/ml) (Table 3). The lower the EC_{50} value, the higher will be antioxidant capacity (Brand-williams *et al.,* 1995, Kahl and Kappus, 1993). Scavenging activity was expressed as EC_{50} (effective concentration in l µg/mL of samples or positive control that reduces the absorbance of DPPH, ABTS by 50% when compared with control) (Iqbal *et al*., 2015).

It is unprecedented that this type of study not done extensively on Andhra pradesh tribal formulation hitherto by any researcher as per literature, as well this study is also the first to report the evaluation of antioxidant compounds and testify after assessing antioxidant capacity for *Nitya Yevvana Kashayam* which is concurrent botanical product claimed and prescribed the tribal health care providers in the forest patches of Talakona. Furthermore, this study served the scientific basis for validating an ethnomedicinal drug and extensive *in-vitro* studies has to be done in the present scenario as an alternate for synthetic Antioxidants.

Conclusion

Nitya Yevvana Kashayam (NYK), an ethnobotanical formulated syrup prescribed for Antidote for all the uneasy and ill health conditions, immune boosting and for strength tested for its presence of antioxidant compounds and antiradical sca-

venging potency which has been successfully proved and supports ethnic claims as a good nutritional and immune boosting supplement for health benefits.

Acknowledgement

The authors acknowledge Department of Biotechnology, Dravidian University, Kuppam for providing facilities to conduct the studies and other logistics provided for this research.

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Cite this article as:

Sripriya D. and M. Chandra Sekhara. Evaluation of Radical Scavenging Activity of Ethnomedicinal Syrup "*Nitya Yevvana Kashayam*". *Annals of Plant Sciences.* 9.8 (2020) pp. 3970-3975.

http://dx.doi.org/10.21746/aps.2020.9.8.2

Subject Editor: Dr. Sateesh Suthari, Telangana, India.

Source of support: Nil; **Conflict of interest:** Nil.