

ANTIOXIDANT PROPERTY ANALYSIS OF TOBACCO LEAVES IN AYURVEDIC FORMULATIONS

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ABSTRACT

The aim of this research is to determine H₂O₂ radical scavenging and total antioxidant activity (using Rutin) with water and ethanolic extracts of an Ayurvedic preparation of *Nicotinatobacum* (Black tobacco leaves). Latest use of tobacco leaves is in the field of neurodegenerative diseases such as Parkinsons disorder. In this study, ethanolic and water extracts were prepared from powdered *N. tobacum* leaves and its Ayurvedic formulations. Antioxidant activities were measured by Rutin method, H₂O₂ radical scavenging assays with UV-Vis spectrophotometer. In conclusion, the Ayurvedic formulations of *N. tobacum* had significant H₂O₂ radical scavenging activity and total antioxidant activity. The results of this study show that the water and ethanol extracts of these alternative formulations of *N. tobacum* can be used as easily accessible source of natural antioxidants and be used in the pharmaceutical industry.

Keywords: *Nicotinatobacum*, Tobacco, Ayurveda, Antioxidant, Rutin, H₂O₂

INTRODUCTION

Tobacco is a product processed from the dried leaves of plants in the genus *Nicotiana* of the family Solanaceae (1,2). The earliest therapeutic applications of tobacco were in general bodily ills, catarrh, colds, and fevers, as an aid to digestion and in prevention of hunger and thirst, as a purgative and as a narcotic. Later in the twentieth century, attention switched to diseases affecting the brain and nervous system and studies show that post-encephalitic Parkinsonism can be treated with subcutaneous injections of nicotine. Chemical characteristics of *N. tobacum* include major alkaloids such as nicotine, nor nicotine, anabasin etc. Some of the reactive oxygen species, including hydrogen peroxide, singlet oxygen, hydroxyl and superoxide radicals, have positive roles in energy production

in vivo systems, phagocytosis, intercellular signal transfer, regulation of cell growth and the synthesis of important biological compounds (3,4) Additionally, reactive oxygen species modify DNA and membranes by attacking the lipids, proteins, and carbohydrates in cell membranes and tissues (5).

In the organism, the rates of production and removal of free radicals are in balance, known as oxidative balance. An increase in the rate of production or a decrease in the rate of removal disrupts this balance and increases the levels of reactive oxygen species. This condition, which is called oxidative stress, indicates a serious imbalance between the production of free radicals and the antioxidant defense systems, resulting in tissue damage (6).

Rutin, a well-known natural antioxidant, is one of the medicinally important flavonoids found in tobacco. It makes up to 1% of the whole dried tobacco plant (7). Rutin can reduce capillary fragility, swelling and bruising and has been used in the treatment of venous insufficiency (varicose veins, haemorrhoids, diabetic vascular disease, and diabetic retinopathy), and for improving micro-vascular blood flow (pain, tired legs, night cramps, and restless legs) (8,9)

The formulation of Tobacco mashi is a well-known ayurvedic formulation and is official in the Ayurvedic Formulary of India (10), and traditionally used for asthma, cough and cold, other respiratory disorders. Though it is a very popular medicine, no establishment of quality control for this drug studies have been performed yet. This paper reports H₂O₂ radical scavenging and total antioxidant activity using rutin of the Ayurvedic alternative formulations of *N. tobacum* leaves by instrumental methods to ensure the identity, Potency, Purity, Safety and efficacy of the two types of Ayurvedic formulations; namely Tobacco Formulation 1 (TF1) and Tobacco Formulation 2 (TF2).

METHODS AND MATERIALS

Procurement Of Raw Materials And Formulation:

Nicotina Tobacum leaves were locally procured in Pune, India. For extraction (ethanol or water), 5g powder of the tobacco samples (ayurvedic preparations) were ground into a fine powder in a mill and were mixed in a 50:50 solvent system of ethanol: glacial acetic acid (15mL per sample). Extraction continued until the extraction solvents became dark in colour but clear. The obtained extracts were filtered over Whatman No. 1 paper and the filtrate was collected, then solvent was removed by a rotary evaporator at 50°C (11).

Estimation Of Physicochemical Properties:

It was observed that both the formulations possessed a dark colour and lower density as compared to the crude tobacco. Also, it was observed that the characteristic nicotinic odour of crude drug was lost after the formation, increasing its pharmaceutical palatability. After

macerating in an organic solvent system of ethyl acetate and ethanol, the formulations were found to impart a darker colour to the solvent system, possibly signifying activation of the alkaloids. On vigorous shaking with an organic solvent, the crude drug shows no foaming. However, both the formulations showed foaming, showing the activation of saponins. The test for carbohydrates using Molisch reagent and heating the extract showed a violet-blue colouration with the crude tobacco but showed no change in colour in the formulations, showing an inactivation of carbohydrate moieties.

Hydrogen Peroxide Scavenging Capacity:

The ability of the *N.tobacum* extracts to scavenge hydrogen peroxide was determined (12). A solution of hydrogen peroxide (40 mM) (13) was prepared in phosphate buffer (pH 7.4). Both extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide.

Antioxidant Analysis With Rutin:

The ability of the formulation to show antioxidant activity was determined using the Rutin method (14). The drug powder was macerated with methanol and filtered through Whatmann filter paper. 1ml of this filtrate was taken and mixed with 1ml 2% solution of AlCl₃ and then diluted with methanol to make up the volume to 10ml. The solution was incubated for an hour and absorbance measured at 415nm using rutin as the standard. Methanolic solution of Rutin was prepared by diluting 0.01mg in 1ml.

RESULTS AND DISCUSSIONS

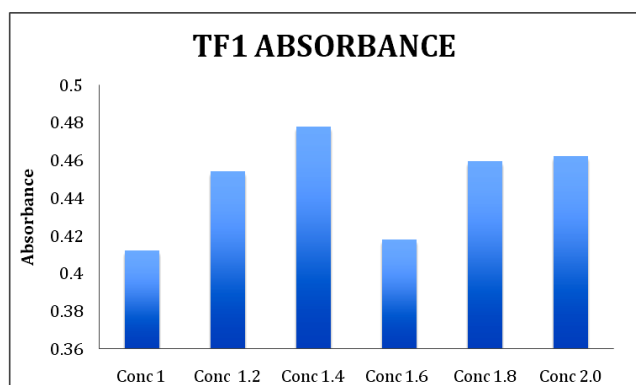
Hydrogen Peroxide Scavenging Property:

The antioxidant properties of Formulation 1 (TF1) and Formulation 2 (TF2) are seen in the graphs 1-2.

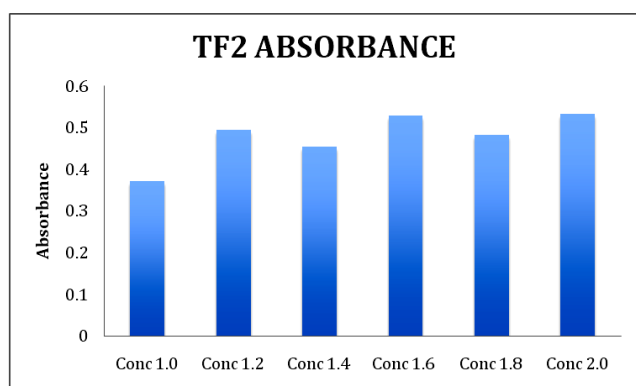
In both the formulations, a linear graph is seen, indicating that there is linear relationship between the concentration and the scavenging

property showing that as one increases the concentration, a potential increase in scavenging abilities is seen.

Graph-1 Antioxidant properties (H₂O₂) of Formulation 1 (TF1)



Graph-2 Antioxidant properties of Formulation 2 (TF2)



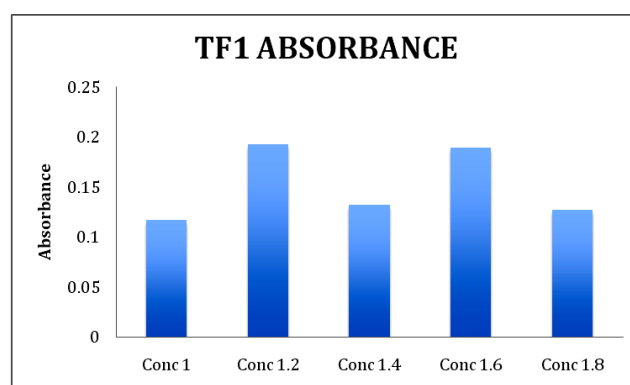
In TF1, a linear increase is seen up to concentration of 1.4, after which a gradual drop is seen till 1.6 and again a linear increase is seen for the upper concentrations. This indicates that an increase in antioxidant properties is seen up to 1.4, a slight drop at 1.6 and a significantly high activity is seen 1.6 onwards again. In TF2, a more uniform graph is seen with only a slight drop in antioxidant activity at around 1.4 and again picking up on higher concentration. From these graphs, it can be inferred that as we progress with dilutions, the AVC of H₂O₂ scavenging activity till the concentration of 1.6 is ellipsoidal which indicates rhythmic successive decline in activity. Above 1.6, the graphs become arrhythmic. If peak plasma levels are studied, it can show similar outputs.

The slight change in the antioxidant properties of the two formulations can be explained by the difference in the way the pyrolysis of the tobacco leaves was carried out. Heat was provided in different ways to both, which caused different changes in its chemical moiety, explaining the difference in their scavenging properties.

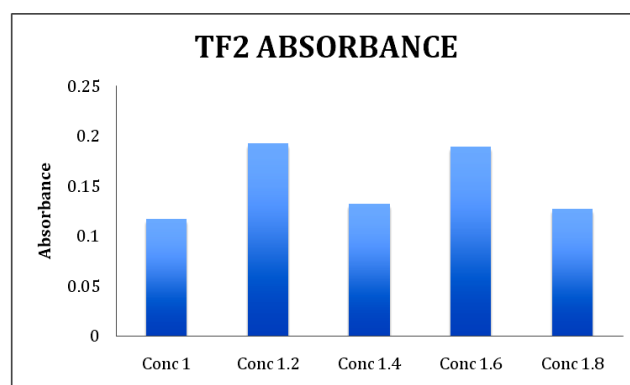
Rutin Analysis:

The antioxidant properties of Formulation 1 (TF1) and Formulation 2 (TF2) are seen in the graphs 3-4.

Graph-3 Antioxidant properties (Rutin) of Formulation 1 (TF1)



Graph-4 Antioxidant properties (Rutin) of Formulation 2 (TF2)



Significant absorbance with UV spectrometer at 415nm was observed for both the formulations, indicating equivalent antioxidant properties. Association and dissociation of the molecule in the solvent system enhances the absorbance at the initial concentration but as the concentration of solute increases in the system a marked decrease is observed, indicating that the system

approaches molecular rearrangement which results in a significant decrease in absorbance.

There is a molecular rearrangement resulting in an improvement of activity with 61.5% per 0.2ml increase in concentration, which further maintains antioxidant levels. Thus, it can be inferred that the antioxidant activity of the formulation which has been formulated using Ayurvedic method has very strong antioxidant properties which can be claimed to be used as an effective anti-bacterial/anti-viral and anti cancer agent with the right titration of doses.

The activities checked were found to be very strong even though the direct formulation was utilized. Thus, if an extract of the formulation was prepared and used, a more significant activity would be observed, likely to be even more than that of Rutin (standard drug).

The drug present in the formulation has already been proven to have three very good antioxidants namely apigenin, quercetin and rutin (15), which are so carried over in the formulation.

CONCLUSION

The grounds of preconceived notions that tobacco is a habit forming drug can be overcome with the type of formulation we have made and can be utilized as a good anti-oxidant drug.

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