***International Journal of Pharmacological Research***
ISSN: 2277-3312 (Online)
Journal DOI: https://doi.org/10.7439/ijpr **Research Article**

# Evaluation of *in-vitro* antioxidant activity of marketed Tobacco products

**Prachi Barsagade***, Rakesh Bhute, A. Y. Sahare and A. O. Maske

*Bajiraoji Karanjekar College of Pharmacy, Sakoli, Dist: Bhandara. Maharashtra-441802, India*

## Abstract

**Objective:** In this study, the antioxidant activities of methanolic extract of marketed tobacco products were evaluated by different *in vitro* antioxidant methods.

**Materials and methods:** The different marketed tobacco products were extracted with methanol by means of maceration process. These extracts were screened for antioxidant activity by different *in vitro* assay methods including Reducing power assay, Phosphomolybdenum assay, Iron chelating assay and Nitric oxide scavenging activity. The screening was carried out at different concentration including 100, 200, and 300µg/ml in reducing power assay, phosphomolybdate assay and Nitric oxide scavenging activity while in chelating assay the extract was used in concentration of 50, 100, 150 and 200µg/ml.

**Results:** All Tobacco products exhibit the antioxidant potential with increasing concentration. The anti-oxidant activity of the marketed tobacco product might be attributed to its polyphenolic content and other phytochemical constituents. Hence, further investigation need to be carried out to isolate and identify the anti-oxidant compounds present in the Tobacco extract.

**Conclusion:** Our study concluded that, the Tobacco causes addiction and dependence but till it has many folklore traditional medicinal uses. If it is used in positive way then it has power to treat and protect but if misused then it have power to harm the body.

**Keywords:** Tobacco products, Reducing power assay, Phosphomolybdate assay, Iron chelating assay, Nitric oxide scavenging assay.

***Correspondence Info:**
Prachi Barsagade
Bajiraoji Karanjekar College of Pharmacy,
Sakoli, Dist: Bhandara
Maharashtra-441802, India

***Article History:**
**Received:** 12/09/2019
**Revised:** 27/10/2019
**Accepted:** 29/10/2019
**DOI:** https://doi.org/10.7439/ijpr.v9i10.5318

**QR Code**

**How to cite:** Barsagade P, Bhute R, Sahare A. Y. and Maske A. O. Evaluation of in-vitro antioxidant activity of marketed Tobacco products. *International Journal of Pharmacological Research* 2019; 09(10): e5318. Doi: 10.7439/ijpr.v09i10.5318 Available from: https://ssjournals.com/index.php/ijpr/article/view/5318



## 1. Introduction

In our body, cells Produces the oxidants in normal and pathological condition. Such Oxidants are useful to our body to destroy the microbes. Sometime the uncontrolled production of oxygen derived free radical such as Reactive Oxygen Species (ROS). This ROS mediated the oxidative damage to micro molecules and it causes the various disease such as Cardiovascular disease, Cancer, Aging, Diabetes, Rheumatoid arthritis, Cirrhosis, etc. [1].

However, antioxidants have evolved with protective roles against such damage. Many medicinal plants have antioxidant value which can prevent the destructive/ degenerative effects caused by oxidative stress [2]. Oxidative stress indicates a serious imbalance between the production of free radical and the antioxidant defence system, resulting in tissue damage [3].

Although Tobacco is well known cancer causing agent many traditional uses claim its medicinal value. The different scientific study reveal the presence of different chemical constituents in tobacco including polyphenol as antioxidants, hence present study was done with the objective to evaluate the antioxidant activity of various Tobacco products which are easily available in market. Tobacco is a product processed from the dried leaves of *Nicotiana Tobacum* (family: Solanaceae) [4,5]. In India,

Tobacco products are more commonly available in local market. The Tobacco plant contains high level of the addictives chemical constituents. The leaves may be smoked (in cigarette, cigar and pipes), applied to the gums (as dipping and chewing Tobacco) or inhaled (as snuff). It causes addiction and dependence and having carcinogenic activity but still it contains the excellent antioxidant potential. Most indigenous nation have traditional studies explaining how tobacco was introduced to their communities, many of which emphasized to sacred properties of plants, containing both the power to heal if used properly and the power to cause harm if used improperly [6]. The scientific investigation indicates the presence of phenolics and flavanoid constituents in tobacco [7] and medicinal plants containing such constituents can express antioxidant potential [8].

In this study, in-vitro antioxidant activity of methanolic extract of marketed tobacco products were evaluated by various methods including Reducing power assay, Phosphomolybdenum assay, Iron chelating assay and Nitric oxide scavenging activity. It has been used as one of the antioxidant capability indicators of medicinal herbs [9].

Reducing properties are generally associated with the presence of reductones, which are believed to break radical chain and the donation of H atom indicting the antioxidant properties of plants concomitant with the development of reducing power. The higher the absorbance, the stronger the reducing power [10]. Phosphomolybdenum method is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo(VI) to Mo(V) by the sample analyte and subsequent formation of a green phosphate Mo(V) complex at acidic pH [11]. Iron chelating activity assay ortho substituted phenolic compounds may exert prooxidant effects by interacting with iron. O-phenanthroline quantitatively forms complexes with Fe2+, which get disrupted in the presence of chelating agents. [12].

Nitric oxide is an essential bio-regulatory molecule required for several physiological processes like neural signal transmission, immune response, control of vasodilatation and control of blood pressure etc. [13]. However the elevation of NO result in several pathological conditions including cancer. Moreover in the pathological conditions nitric oxide react with superoxide anion and form potentially cytotoxic molecules, peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspects of inflammation and tissue damage seen in inflammatory diseases [14]. The level of nitric oxide was significantly reduced in this study by the methanolic extract. Since NO play a crucial role in the pathogenesis of cancer this may explain the use of Tobacco for the treatment of cancer [15].

## 2. Material and Methods

### 2.1 Collection of Sample:

All chemicals and reagents used in the study were of analytical grade and were procured from Department of Pharmaceutical Chemistry, Bajiraoji Karanjekar College of Pharmacy Sakoli, Bhandara, Maharashtra, India. Chewing Tobacco products were purchased from the local market of Sakoli. The four different Tobacco product with the brand name including Thawkar (S1), Mazza (S2), Janam (S3) and Eagle (S4) were collected in the month of June 2018.

### 2.2 Preparation of Extract:

The coarsely grounded tobacco products were macerated separately with methanol. The maceration process was allow for 15 days in tightly sealed vessels at room temperature and stirred several time daily. After 15 days, the mixtures were filtered through muslin clothe and thereafter concentrated by evaporation at room temperature.

### 2.3 Determination of Antioxidant Potential:

#### A. Reducing Power Assay:

The reducing Power of methanolic extract of tobacco products was determined by the method prescribed by Oyaizu [17]. The various concentration of Tobacco in methanol was mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50°C in water bath for 20 min. After cooling, 2.5 ml of 10% trichloroacetic acid was added and centrifuge at 3000 rpm for 10 min whenever necessary. The 2.5 ml of upper layer of solution was mixed with 2.5ml of distilled water and 0.5 ml of freshly prepared ferric chloride solution. The blank was prepared in similar manner excluding sample. The absorbance was measured at 700 nm. Ascorbic acid at various concentrations was used as standard. Increase absorbance of the reactions mixture indicates increase in reducing power.

#### B. Phosphomolybdenum Reduction Assay:-

The total antioxidant capacity of the methanol extract was evaluated by the Phosphomolybdenum reduction assay method according to the procedure described by Prieto et al. [18]. The assay is based on the reduction of Mo (VI) to Mo (V) by the methanol extract and subsequent formulation of green phosphate/ Mo (V) complex at acid pH. The 1 ml of various concentrations (100-300ug/ml) of extract was mixed with 1ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate) and incubated at 95°C for 90 min. The absorbance of the reaction mixture was measured at 765nm using a UV-visible spectrophotometer. Ascorbic acid was used as reference standard.

**C. Iron Chelating Assay:-**

The reaction mixture containing 1ml o-phenanthroline, 2ml Ferric chloride solution and 2ml extract at various concentrations (50-200 µg/ml) in final volume of 5ml was incubated for 10 min at ambient temperature and the absorbance was recorded at 510 nm. Ascorbic acid as standard drug was added instead of extract and absorbance was taken as equivalent to 100% reduction of all ferric ions. Blank was carried out without drug [19].

**% chelating activity =**

**{1- absorbance (T)/ Absorbance (B)} × 100**

Where, Absorbance (T): Absorbance of test

Absorbance (B): absorbance of Control

**D. Nitric Oxide Scavenging Activity:-**

Nitric oxide scavenging activity was measured by using Griess' reagent. The 2 ml of 10 mM sodium nitroprusside in standard phosphate buffer (pH 7.4) was mixed with 0.5 ml of extract at various concentrations (100-400 ug/ml) and the mixture was incubated at 25°C for 150 minutes. The 0.5 ml of incubated solution was mixed with 1ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and incubated at room temperature 25°C for 5 min. Finally, 1ml of Napthylethylene diamine dihydrochloride (0.1% v/v) was added and incubated at room temperature for 30 minutes. The absorbance was measured at 540 nm by using UV-visible spectrophotometer [20].

**% Nitric oxide inhibitor activity= (Ao – As/ Ao) × 100**

Where, Ao= Absorbance of control

As = absorbance in the presence of the extract.

## 3. Result and Discussion:

The antioxidant activity of methanol extract of marketed prepared tobacco sample was evaluated by reducing power assay, phosphomolybdate assay, o-phenanthroline method and nitric oxide radical scavenging method.

Reducing power assay of the methanolic extract of marketed product tobacco showed almost similar increasing trend in reducing power with the increased extract concentration. In this assay, the presence of reducers as an antioxidant causes the reduction of the ferric to the ferrous form and the colour of the test solution change to various shades of green and blue depending upon the reducing power of extract. The data present in the figure indicate the antioxidant activity of different extract of Tobacco product which is due to presence of reductones by donating the electrons and reacting with free radical to convert them to more stable product and terminate free radical chain reaction [21]. Phosphomolybdate assay involve the measurement of reduction degree of Mo (VI) to Mo (V). Phosphomolybdate assay is a quantitative method to investigate the reduction reaction rate among antioxidant, oxidant and molybdenum ligand. It involves in thermally generating auto oxidation during prolonged incubation period at higher temperature. It gives direct estimation of reducing capacity of antioxidant. In Iron chelating assay, the extract interfere with the formation of ferrous-o-phenanthroline complex, thereby suggesting that the extract has metal chelating activity. Iron stimulates lipid peroxidation by Fenton reaction and accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals which themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. The metal chelating capacity is important, since it reduces concentration of the catalyzing transition metal in lipid peroxidation. It has been reported that the chelating agents, that form bonds with a metal are effective as secondary antioxidant because they reduce the redox potential, thereby stimulating the oxidized form of the metal ion. The observed results demonstrate a marked capacity of the extract for iron binding, suggesting that their action as a peroxidation protector may be related to its iron binding capacity. Nitric oxide (NO) is a reactive free radical produced by phagocytes and endothelial cells to yield more reactive species such as peroxynitrite which can be decomposed to form OH radical. In this study, level of nitric oxide was significantly reduced by extract, explaining its role as antioxidant. NO scavenging capacity of the extract may help to arrest the chain of reactions initiated by excess generation of NO that lead to various pathogenic pathways underlying a large group of disorders including muscle diseases, inflammatory bowel disease, sepsis and septic shock, primary headaches and stroke.

**Table 1: Reducing Power Assay**

| Concentration | S1 | S2 | S3 | S4 | AS |
|---|---|---|---|---|---|
| 100 | 0.117 | 0.06 | 0.083 | 0.021 | 0.048 |
| 200 | 0.105 | 0.05 | 0.107 | 0.027 | 0.166 |
| 300 | 0.145 | 0.135 | 0.112 | 0.094 | 0.28 |

S: Sample, AS: Ascorbic acid.

**Table 2: Phosphomolybdate assay**

| Concentration | S1 | S2 | S3 | S4 | AS |
|---|---|---|---|---|---|
| 100 | 0.251 | 0.257 | 0.162 | 0.235 | 0.607 |
| 200 | 0.411 | 0.409 | 0.376 | 0.463 | 1.15 |
| 300 | 0.571 | 0.588 | 0.423 | 0.593 | 1.676 |

S: Sample, AS: Ascorbic acid.

**Table 3: Iron Chelating Activity Assay:-**

| Concentration | S1 | S2 | S3 | S4 | AS |
|---|---|---|---|---|---|
| 50 | 0.55 | 0.55 | 0.67 | 0.57 | 0.57 |
| 100 | 0.56 | 0.55 | 0.62 | 0.58 | 0.56 |
| 150 | 0.57 | 0.57 | 0.64 | 0.58 | 0.54 |
| 200 | 0.58 | 0.58 | 0.61 | 0.59 | 0.53 |

S: Sample, AS: Ascorbic acid.

**Table 4: Nitric Oxide Scavenging Activity:-**

| Concentration | S1 | S2 | S3 | S4 | AS |
|---|---|---|---|---|---|
| 100 | 5.02 | 2.79 | 1.11 | 0.55 | 1.98 |
| 200 | 7.26 | 2.79 | 6.14 | 1.11 | 5.36 |
| 300 | 7.26 | 3.35 | 7.26 | 1.67 | 9.25 |
| 400 | 15.64 | 3.91 | 8.93 | 3.35 | 12.6 |

S: Sample, AS: Ascorbic acid.

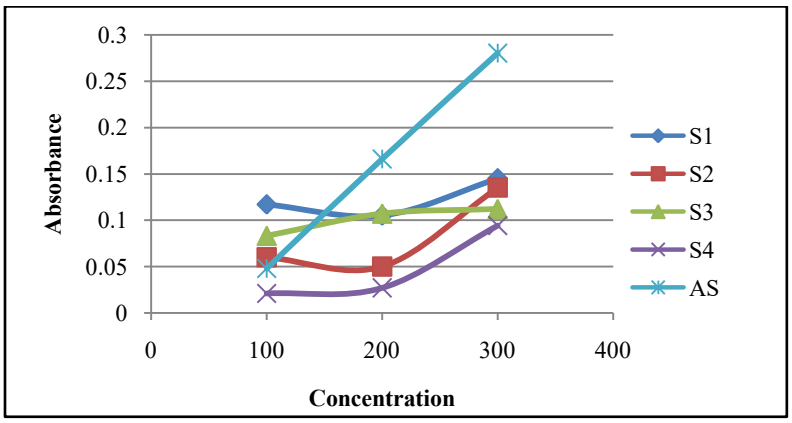


**Figure 1: Reducing Power Assay**

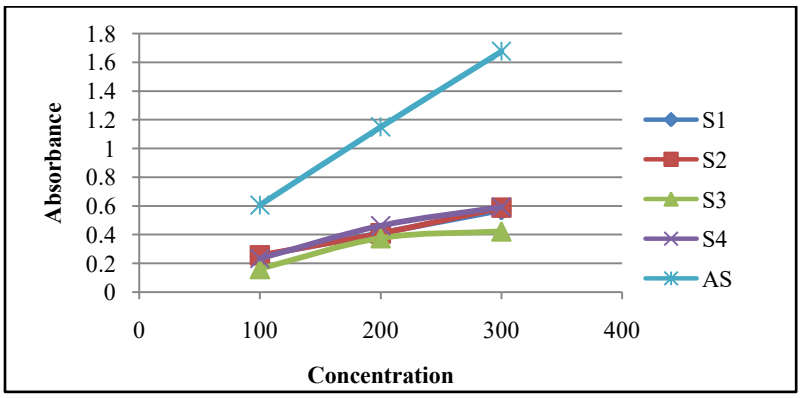


**Figure 2: Phosphomolybdate Assay**

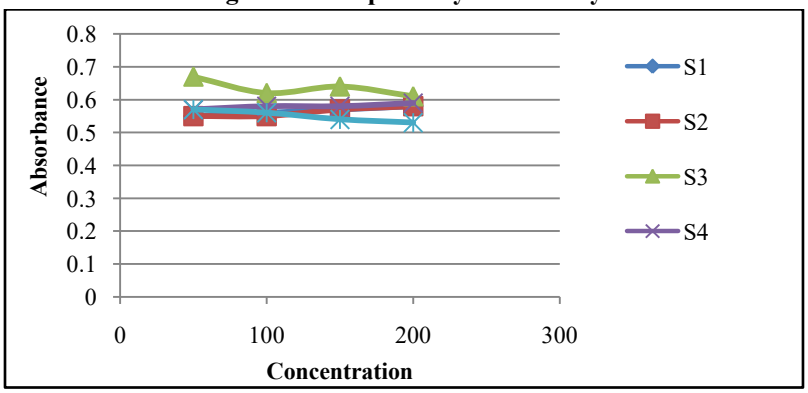


**Figure 3: Iron Chelating Activity Assay:-**

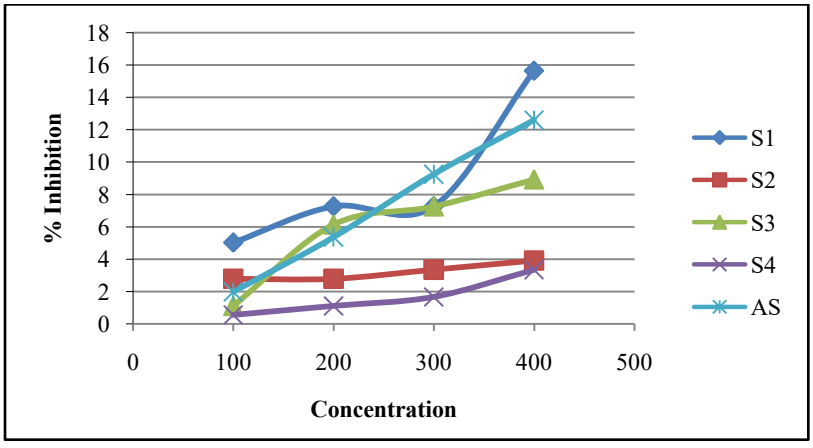


**Figure 4: Nitric Oxide Scavenging Activity**

## 4. Conclusion

Tobacco causes addiction and dependence but till it has many practical folklore traditional medicinal uses. If it is use in positive way then it had the power to heal and protect but if misused then it had the power to harm.

From the results obtained in the present study it is concluded that a methanolic extract of Tobacco products, which contain phenolic compounds, exhibits antioxidant and free radical scavenging activity. These assays indicate that tobacco may contain antioxidants components, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigation need to be carried out to isolate and identify the antioxidant compound present in the extract.

## References


[1]. Polterat O., Antioxidants and Free Radical scavengers of natural origin, *Current Org. Chem.* 1997; 1; 415-440.

[2]. Olugbami J. O., Gbadegesin M. A., and Odunola O. A., In-vitro free radical scavenging and antioxidant properties of ethanol extracts of *Terminalia Glaucescens*, *Pharmacognosy Res.* 2015; **7**: 49-56.

[3]. Wells P.G., McCallum G.P., Chen C. S., Henderson J.T., Lee C. J., Perstin J., Preston T. J., Wiley M. J., and Wong A. W., Oxidative stress in developmental origins of disease: Teratogenesis, Neuro developmental deficit and cancer, *Toxicological science,* 2009; 108(1): 4-18.

[4]. Evans W., Trease and Evans, Pharmacognosy. 14th ed. WB Saunder Company Ltd, London. 1997; Vol 16(3): 182-183.

[5]. Mukherjee P., Quality Control of Herbal Drugs: An approach to evaluation of Botnicals, 5th reprint. 2012.

[6]. Binorkar S. V., Jani D. K., Traditional Medicinal usage of Tobacco A Review, Spatula DD, 2012; Vol. 2: 127-134.

[7]. Nasr S. B., Mnif S. A. W., Miguel M., Phenol Content and Antioxidant Activity of Different Young and Adult Plant Parts of Tobacco from Tunisia, dried at 40°C and 70°C. *J. of applied Pharmaceutical Science*, 2014; 4(8): 23-31.

[8]. Wantha S., A review an antioxidant methods. *Asian J. Pharmaceutical and Clinical Research* 2016; 9(2): 14-32.

[9]. Neelam S. I. A., Hany O. E., and Jabeen S., Antibacterial and Antioxidant Effect of Chewable Tobacco Leaf Product (Patti) and Bettel nut an Environmental Pathogens, *Int. J. Biol. Res.* 2013; 1(2): 133-135.

[10]. Kumar S., Analytical techniques for Natural product Reserch, CABI, Vol.171; 2015.

[11]. Kanner J., Natural antioxidants in grapes and wines. *J. Agric. Food. Chem.* 1994; 42: 64-69.

[12]. Shreedhara C.S., Aswatha Ram H.N., Sachin B. Z., and Falguni P. G., In vitro antioxidant potential of ethanolic extract of *Momordica dioica* roxb (Cucurbitaceae). *Pharmacologyonline*, 2011; 3: 622-633.

[13]. Rees D.D., Palmer R. M., and Moncada S., Role of endothelium derived nitric oxide in the regulation of blood pressure. Proceedings of the National Academy of Sciences USA, 86; 1989; 3375-3378.

[14]. Shaligram V.L., and Nighantubhushanam S., Khemraj Krishnadas Prakashan Mumbai. 4th Edition, 908; 2004.

[15]. Bapalal G.V., and Adarsha N., Chaukhambha Bharti Academy. 2009; Vol. 2; 146.

[16]. Kishore K., review article Monograth Of Tobacco (*Nicotana Tobacum*) Department of Pharmacy, M.J.P. Rohilkhand University, Barelily, U.P., India, *Indian Journal of Drugs*, 2014; 2(1): 5-23.

[17]. Oyaizu M. Studies on products of browning reaction. *The Japanese journal of nutrition and dietetics*. 1986; 44(6): 307-15.

[18]. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*. 1999 May 1; 269(2):337-41.

[19]. Rana M. G., Katbamna R. V., Padhya A.A., Dudhrejiya A. D., Jivani N.P., Sheth N. K., *In vitro* antioxidant and free radical scavenging studies of alcoholic extract of *Medicago Sativa* L. *Romanian J. of Biology – Plant Biology*, 2010; 55(1): 15-22.

[20]. Marcocci L., Maguire J. J., Droylefaix M. T., Packer L. The nitric oxide scavenging properties of *Ginkgo biloba* extract EGb 761. *Biochem. Biophys. Res. Commun.* 1994; 201: 748-755.

[21]. Jayaprakasha G. K., Selvi T., Sakariah K. K., Antibacterial and antioxidant activities of Grapes. (*Vitis Vinifera*) seed extracts. *Food Research International.* 2003; 36(2): 117-122.

[22]. Ross R., The pathogenesis of atherosclerosis: a perspective for the 1990's. *Nature*, 1993; 362: 801.