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Original Article

EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITY OF "*SMRITHI*", A POLY HERBAL FORMULATION

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ABSTRACT

The study is an attempt to investigate antioxidant activity of ethanolic extract of polyherbal formulation of plants *Bacopa monniera, Centella asiatica, Acorus calamus, Emblica officinalis, Asparagus racemosus*(*smrithi)*were prepared and analyzed for phytochemical analysis and *in-vitro* antioxidant activity. *In-vitro* anti-oxidant activity was studied by using DPPH(1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging method and H_2O_2 hydrogen peroxide method using ascorbic acid as standard. In the present study, the extract of polyherbal formulation was found to possess good anti-oxidant activity. The activity of polyherbal formulation extract may be attributed to their free radical scavenging ability, the extract of antioxidant activity of "*smrithi"* was found significant.

INTRODUCTION

In the last few years, there has been a significant growth in the arena of herbal treatment. It is getting propagated in the emergent countries due to its natural origin and lesser side effects [1]. It is categorized in the olden Indian system of medicine (Ayurveda) as Rasayana, a group of plant derivatives remedies that develop overall physical and mental health and put off diseases by rejuvenating the body in incapitated conditions [2].

An antioxidant is defined as a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions [3]. They do so by being oxidized themselves; so antioxidants act often as reducing agents such as thiols, ascorbic acid or poly phenols. Oxidative-process is the most common route for producing free radicals in food, drugs and even in living systems [4]. Free radicals contribute to different human disorders like atherosclerosis, arthritis and ischemia and reperfusion injury of many tissues gastritis, cancer. The majority of free radicals that damage biological systems are oxygen radicals.

Antioxidants also act as radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors and metal-chelating agents [5]. Due the effect on immune system, there is a need for natural antioxidants (safe and non-toxic) as compared to synthetic antioxidants (toxic for human).

To support the usage of selected plant extracts in Ayurveda, the antioxidant potential of *smrithi* formulation of *Bacopa monniera, Centella asiatica, Acoruscalamus, Asparagus racemosus, Emblica officinalis* [6]. The objective of this work was to assess the antioxidant activity of plant extract of above herbs by *in vitro* studies and relate them with ascorbic acid, a known antioxidant.

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MATERIALS AND METHODS

Chemicals and Reagents

The 2Mm H_2O_2 solution was prepared by dissolving 0.022ml of 30% H_2O_2 in 100 ml distilled water. The 500uM solution of DPPH was prepared by dissolving 23mg of DPPH in 100ml of distilled water. TRIS[2-amino-2(hydroxyl methyl) propane 1-3di-ol] buffer (pH 7.4) was prepared by adding 0.605g of TRIS buffer in 30 ml of water and adding 0.33 ml of concentrated hydrochloric acid, diluted to 100 ml with distilled water. TRIS buffer prevents the sudden pH change during the preparation of test dilutions.

Polyherbal formulation *smrithi*

The poly herbal formulation "*smrithi"* under research was obtained as gift sample from IMIS Pharmaceutical PVT LTD.Aboutfifty grams of *smrithi*was subjected to ethanolic extraction by hot percolationmethod through soxhlet apparatus. The extract was concentrated to semi solid mass. These extract were used for the further studies.[7, 8]

Preliminary phytochemical analysis

The preliminary phytochemical studies were conducted on the active extracts using standard procedures adopted by Harborne (1973) and Gibbs (1974). Preliminary phytochemical analysis on plant extracts was performed using the following chemicals and reagents: flavonoids (Mg turnings and HCl), phenolics ($FeCl_3$), protein and amino acid (Millon's and Ninhydrin reagent), alkaloids (Mayer and Dragendorff's reagent), saponins (Foam test), phytosterols, triterpenoids (Liebermann- Burchard Test),glycosides(killer killani test),tannins(lead acetate solution) and carbohydrates (Fehling's solution A and B)[9, 10, 11]

Preparation of sample solution and dilutions

The stock solution was prepared by dissolving 1 mg of *smrithi* extract into water and make up the volume to 10 ml with water. Prepared the initial dilutions from stock solution using various concentrations of 10, 20, 40, 60,80, 100µg/ml were prepared. In the same procedure ascorbic acid as standard were also prepared at 10, 20, 40, 60, 80,100 µg/ml concentrations.[12]

Determination of antioxidant activity

Evaluation of antioxidant activity by following methods

Free radical scavenging activity (DPPH method)

Free radical scavenging of this formulation by DPPH was estimated accordingto Manzocco *et al*[13]. Series of different concentrations of *Smrithi* were prepared ranging from 10 -100 µg/ml. Freshly prepared 500uM, 1 ml solution of DPPH was taken into 5 ml volumetric flasks, added 0.5 ml of TRIS Buffer and 0.5 ml of serial dilutions of different concentrations (10µg/ml to 100µg/ml) of *Smrithi*. The final volume to 5ml with distilled water. The absorbance of all dilutions were taken after 30minutes at 517nm using a spectrophotometer. Ascorbic acid was used as standard for this assay. The mixture was incubated at 37^0C for 30 minutes.

The capability of the formulation to scavenge the DPPH radical was calculated by using the formula-

Percentage inhibition= (AC-AS)/AC X 100

Where, AC is absorbance of control;

AS is the absorbance of sample

Scavenging of hydrogen peroxide

The ability of plant extract to scavenge hydrogen peroxide can be estimated according to the method of Ruch *et al* [14] 2mM H_2O_2 solution was prepared by dissolving 0.022 ml of 30% H_2O_2 in 100 ml of distilled water. Different concentrations of *smrithi* were prepared 10 -100 µg/ml.2 ml solution of each concentration was mixed with 0.6ml of H_2O_2 solution. After 10 minutes, the absorbance was measured at 230 nm against blank solution. Ascorbicacid was used as a standard. The percentage inhibition was calculated according to the following equation:

Percentage inhibition= (AC-AS)/AC X 100

Where, AC is absorbance of control;

AS is the absorbance of sample

RESULTS

In-vitro antioxidant assay of the polyherbal compound *smrithi* revealed the presence of antioxidant potential. The percentage of inhibition was observed that free radicals were scavenged by the test compounds in a concentration manner in both the methods.The table 1 depicts the results of phytochemical analysis were observed that the presence of flavonoids, phenols, tannins, alkaloids,steroids, carbohydrates.

Table 1: Phytochemical results of smrithi

S.no	Phyto constituents	Ethanolic extract of *smrithi*
1.	Alkaloids	+
2.	Tannins	+
3.	Flavonoids	+
4.	Carbohydrates	+
5.	Steroids	+
6.	Phenols	+
7	Glycosides	-
8	Proteins	-

The DPPH and H_2O_2 scavenging activities were recorded in terms of percentage inhibition observed from table 2 and table 3, that the*smrithi* has maximum DPPH and H_2O_2 scavenging activities (77.6%& 79.3%). The results obtained were comparative to standard ascorbic acid. Higher the percentage inhibition indicates better scavenging activity or antioxidant potential. The results obtained were statistically significant with $p<0.05$.

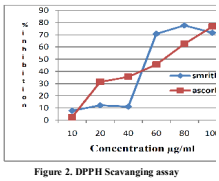

Figure 2. DPPH Scavanging assay

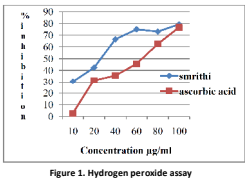

Figure 1. Hydrogen peroxide assay

Table 2: DPPH scavenging activity of Smrithi

S.No	Concentration (µg/ml)	*Smrithi*	%Inhibition	Ascorbic acid	%Inhibition
1.	10	0.922	7.8%	0.973	2.7%
2.	20	0.878	12.2%	0.686	31.4%
3.	40	0.889	11.1%	0.645	35.5%
4.	60	0.293	70.7%	0.644	45.6%
5.	80	0.224	77.6%	0.375	62.5%
6.	100	0.284	71.6%	0.233	76.7%

Table 3: Hydrogen peroxide scavenging activity of smrithi

S.No	Concentration µg/Ml	*Smrithi*	%Inhibition	Ascorbic Acid	% Inhibition
1.	10	0.697	30.3%	0.970	2.7%
2.	20	0.576	42.4%	0.682	31.4%
3.	40	0.335	66.5%	0.640	35.5%
4.	60	0.250	75%	0.620	45.6%
5.	80	0.267	73.1%	0.370	62.5%
6.	100	0.207	79.3%	0.230	76.7%

DISCUSSION

In general, it can be concluded that the antioxidant activity of polyherbal extract shows the presence of alkaloids, tannins, carbohydrates, steroids and flavonoids[14]. In the present study different chemical aspects were evaluated for *in vitro* antioxidant assay of polyherbal formulation.

In living systems, free radicals are constantly generated causing extensive damage to tissues and biological molecules which may lead to various diseases[15]. Many synthetic antioxidant drugs are available but,because of their adverse side effects an alternative solution to this problem is to develop such as natural antioxidants through food supplements and traditional medicines. However, contemporary medicines are useful in the prevention and management of diseases but wide range of side effects and, on the other hand, resistances to antibiotics compels us to search some new natural compounds [16]. The ethanolic extract of *smrithi* may have phenolic and flavonoid content [17]. Were the polyherbal formulation *smrithi* revealed synergistic effects both in DPPH scavenging and hydrogen peroxide methodwhen compared with the literature search of individual plants[18].

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH, transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1 - 1 diphenyl - 2 - picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug[19]. In the present study, the ethanolic extract of-*smrithi* had significant scavenging effect on the DPPH radical which was increasing with the increase in the concentration of the sample from 10 - 100 µg/ml. The percentage of inhibition of the DPPH radical was varying from .7.8% (in 10 µg/ml of the extract) to 71.6% (in 100 µg /mL 0f the extract).

H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells[20, 21].The results showed that extracts of *smrithi* had an effective H_2O_2 scavenging activity. The percentage of inhibition of the H_2O_2 was varying from 30.3% (in 10 µg/ml of extract) to 79.3% (in 100 µg/mL extract).

In conclusion, the data obtained in the present investigation suggest that polyherbal compounds may be good sources of antioxidants for radical scavenging. The highly positive correlation of radical scavenging activity in polyherbal compounds indicates that phenols and flavonoids are important components which are mainly responsible for the free radical scavenging activity.

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REFERENCES

1. Khopde .SM, Priya Darshini, Mohan H, Gawandi VB, Characterizing the antioxidant activity of amla (*Phyllanthus emblica*) extract, curro scist.2001;185-190.
2. Halli well,Gutteridge JMC, Free radicals in biology and medicine os'ed/clarendon.press.oxford.
3. Gutteridge JM, Free radicals and antioxidants in the year 2000. A historical book to the future, Ann N Y, Acad sci 2000,899.
4. Halli well B.Free radicals, antioxidant and human disease: curiosity,cause or consequence?Lancet 1994; 44:721-4.
5. Young IS and Woodside JV. Antioxidants in health and disease. *J Clin Pathol* 2001;54:176-86.
6. Jyothi Vadthya, satyavati.D; Evaluation of nootropic activity of *Smrithi*, a polyherbal formulation. *The pharma innovation journal* .2014; 3 (3), 33-41.
7. Kokate, C. K. Practical Pharmacognosy, 4th edn. VallabhPrakan, New Delhi. 1994, 179-181.
8. Khandelwal. K.R, Practical pharmacognosy, edition,Niraliprakashan, Pune India Reprint, 2005, 27-35.
9. Harborne JB. Phytochemical methods: A guide to modern technique of plant analysis. London: Chapman & Hill; 1998. 26.
10. Harbone, J.B., Turner, B.L. Plant chemosystematics. Academic press, London. 1984: P: 61-62.
11. Gibbs,R.D.Chemotaxonomy of flowering plants, MC Gill queens university press, Montreal and London .1974.

12. Kamran J.Naquvi, Senahlata Dohare*; In vitro* antioxidant activity of *Asparagus racemoses* roots; *Int .J.Bio medical Res*.2011.2 (4), 228-235.
13. Alam MN, Bristi NJ. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharm J*,2013; 21(2): 143-52
14. Ruch RJ, Cheng SJ.Preventaion of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogen,1989; 10: 1003-1008.
15. Kajaria DK,Gangwar M, Sharma A.K, Tripathi YB.Comparative evaluation of phenol and flavonoid content of polyherbal drugs. *Pharmacology online* 2011; 3:1365-73.
16. Hazra B, Biswas S and Mandal N. Antioxidant and free radical scavenging activity of Spondias pinnata. BMC Complem Altern Med 2008;8;63-72.
17. Divya .K.Kajaria, Mayank Gangwar, Jyothi.S. Tripathi; *In vitro* antioxidant potential and radical scavenging activity of polyherbal drug *Shrishadi*; oxidents and antioxidants in medical science 2012,1(3);225-229.
18. P.Padmanabhan,S.N.Jangle.Evaluation of DPPH radical scavenging activity and reducing power of four selected medicinal plants and their combinations;*Int .J.Pharmaceutical sciences and drug Res*.2012; 4 (2) ;143-146.
19. Sochor J, Ryvolova M, Krystofova O, Salas P, Hubalek J, Adam V, Trnkova L, Havel L, Beklova M, Zehnalek J, Provaznik I, Kizek R. Fully Automated Spectrometric Protocols for Determination of Antioxidant Activity: Advantages and Disadvantages. Molecules 2010; 15: 8618-8640.
20. Sakat S.S., *In vitro* antioxidant and anti-inflammatory Activity of methanol extract of *Oxalis corniculata Linn*. *Int. J. of Pharmacy and Pharmaceutical Sciences*, 2010, 2; 146-155.
21. Indira Priyadarsini, G.H.Naik, Hari Mohan. Evaluating the antioxidant activity of different plant extracts and herbal formulations. 2005, 31; 145-51.

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