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Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF A POLYHERBAL FORMULATION

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ABSTRACT

The study is an attempt to investigate antioxidant activity of ethanolic extract of a polyherbal formulation of three drugs *Bryophyllum pinnatum*, *Syzigium aromaticum* & *Ocimum sanctum* by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method using ascorbic acid as standard. In the present study, the extract of polyherbal formulation (*Bryophyllum pinnatum*, *Syzigium aromaticum* & *Ocimum sanctum*) was found to possess good antioxidant activity. This activity of polyherbal formulation extract may be attributed to their free radical-scavenging ability. The extent of antioxidant activity of polyherbal formulation was found significant.

Keywords: Polyherbal; antioxidant activity; ethanolic extract

INTRODUCTION

In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation. Ethnopharmacological surveys conducted among herbal practitioners of traditional Arab medicine in Palestine and the Middle East have revealed that a large number of indigenous plant species are being used as a source of herbal therapies. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity, including *Ocimum sanctum*, *Piper cubeba* Linn, *Allium sativum* Linn, *Terminalia bellerica, Camellia sinensis* Linn., *Zingiber officinale Roscoe* and several Indian and Chinese plants. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins. [1]

Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer. Spices and herbs are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases and aging. [2]

The plants under study are *Syzigium aromaticum* belongs to family Myrtaceae, traditionally used in the treatment for stomach upsets, vomiting and diarrhea etc. [3] *Bryophyllum pinnatum* belongs to family Crassulaceae traditionally used in the treatment for Tonsillitis, traumatic injury, fracture and strains etc. [4] & *Ocimum sanctum* belongs to family Lamiaceae traditionally used as antiseptic, analgesic and controls the infections. [5]

The present study is to determine the antioxidant capacity of ethanolic extract of polyherbal formulation of three drugs *Bryophyllum pinnatum*, *Syzigium aromaticum* & *Ocimum sanctum*.

MATERIALS AND METHODS

Plant material

The plant parts (leaves of *Bryophyllum pinnatum*, buds of *Syzigium aromaticum* & Leaves of *Ocimum sanctum*) were procured from local market of Bhopal (M.P.) and authenticated from Department of Botany, Saifia College, Bhopal (Voucher No. 277/bio/saf/11/a, 278/bio/saf/11/b, 279/bio/saf/11/c). After authentication the plant parts were washed, shade dried and ground in a mechanical grinder to obtain coarse powder for extraction.

Materials chemicals

DPPH (1, 1-Diphenyl-2-picrylhydrazyl), gallic acid, potassium ferricyanide, phosphate buffer (P_H 6.6), trichloroacetic acid and ferric chloride. All other reagents were of analytical grade obtained from Rajeev Gandhi College of Pharmacy, Bhopal.

Plant extraction

The powdered plant parts were extracted with ethanol using maceration method. The extract was then dried and stored. 0.714 mg of each extract were taken and mixed to prepare a polyherbal formulation for assaying antioxidant activity.

Scavenging effects of plant on DPPH radical

Free radical scavenging effect was determined using the free radical generator DPPH (2,2-diphenyl-1- picrylhydrazyl). Different concentrations of plant extract were prepared in methanol ranging from 25 µg/mL to 250 µg/mL. Standard DPPH solution containing 400 micromole DPPH was prepared in methanol. Standard DPPH solution was then mixed with test drug dilution at a ratio 1:3 i.e. 1mL of test extract was mixed with 3 mL of Standard DPPH solution in different properly closed containers. The mixtures were kept in the dark at a room temperature for 90 minutes. Absorbance of resulting solution was measured using spectrophotometer at 517 nm. [6-10]

Scavenging activity was calculated by using equation:

$$\text{Scavenging activity (\%)} = \left(\frac{\text{1- Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517 nm}} \right)$$

The antioxidant activity is expressed as IC_{50}. The IC_{50} value is the measure of concentration in µg/ml of extract that inhibits 50% of DPPH radicals.

Reducing power of herbal plant extract

The reducing power of nutraceutical herbs was determined according to the method of Oyaizu (1986). Extracts in 1 mL distilled water were mixed with phosphate buffer (2.5 mL, 2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%); the mixture was incubated at 50ºC for 20 min. A portion (2.5 mL) of trichloroacetic acid (TCA, 10%) was added to the mixture which was then centrifuged at 1500g for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl3 (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. [11]

RESULTS AND DISCUSSION

IC_{50} value for ethanolic extract of polyherbal formulation was found to be 21.99 ± 0.212 µg/mL and whereas for ascorbic acid it is 24.48 ± 0.204 µg/mL. Thus ethanolic extract of polyherbal formulation possesses good antioxidant activity as compared with standard (Table 1).

The reducing power of ethanolic extract of polyherbal formulation was studied using potassium ferricyanide reduction method, the amount of Fe2+ complex was then monitored by measuring the formation of Pearl's Prussian blue at 700 nm. Graph 1 shows the reducing power of the test drug extract increased with increase in concentration. Increased absorbance of the reaction mixture indicated the increased reducing power, thus it is clear that

ethanolic extract of polyherbal formulation possess good reducing power. (Table 2)

Table 1: Antioxidant activity by DPPH method

S. NO.	SAMPLE	IC_{50} VALUE (µG/ML.)
1.	Ethanolic extract of polyherbal formulation	21.99 ± 0.212
2.	Ascorbic acid (standard)	24.48 ± 0.204

The data are expressed as mean value ± SD (n =3).
All values are significant at P< 0.05. Calculated using Graph pad (ANOVA)

Table 2: Reducing power method

S. NO.	CONCENTRATION (µG/ML)	POLYHERBAL FORMULATION ABSORBANCE	ASCORBIC ACID ABSORBANCE
1.	20	0.422±0.012	0.33±0.001
2.	40	1.296±0.125	0.47±0.014
3.	60	1.889±0.117	0.62±0.200
4.	80	2.173±0.091	0.74±0.001
5.	100	2.351±0.157	0.82±0.010

The data are expressed as mean value ± SD (n =3).
All values are significant at P< 0.05. Calculated using Graph pad (ANOVA)

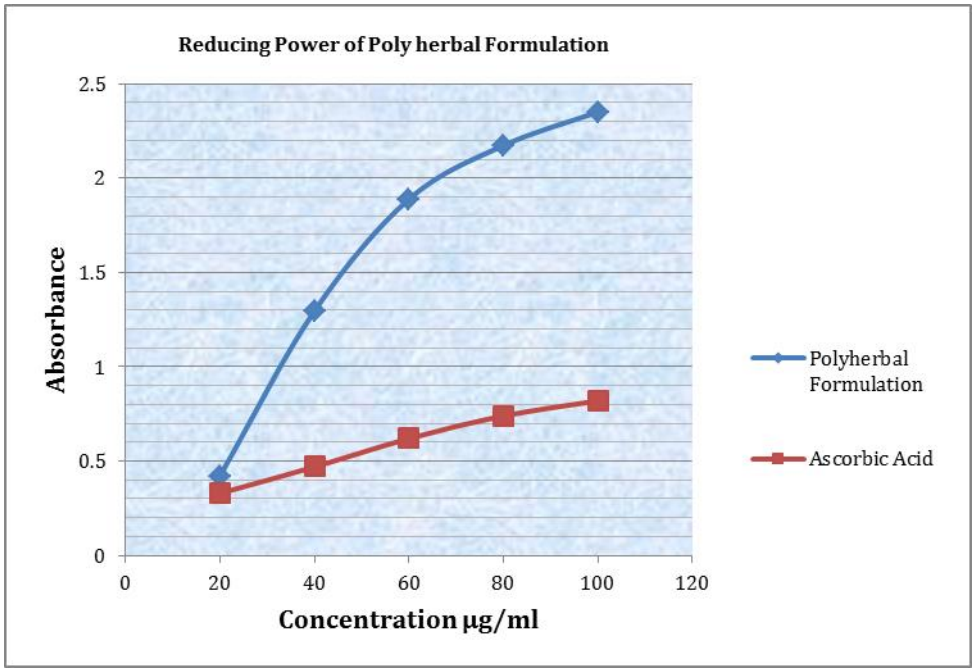

Figure 1: Graph showing reducing power of polyherbal formulation.

It is clear from the above results that ethanolic extract of polyherbal formulation possesses good antioxidant activity.

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