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RESEARCH ARTICLE

In Vitro Anti- Oxidant Study of Herbal Extract Mixture by Nitric oxide and DPPH Method

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ABSTRACT:
Herbalism is a traditional medicine or folk medicine practice based on the use of plants or it's extracts. Herbal extract mixture produce synergetic activity compared to individuals, it was confirmed by its in- vitro antioxidant activity. Herb-herb combinations have been used in practice for thousands of years, yet scientific evidence of their therapeutic benefits is lacking.An increase demand for this , the present work examine thepotential of Herbal extract mixture. Mixtures were prepared in different combination (aqueous extract of *Terminalia belerica* : aqueous extract of *Solanum xanthocarpum*) ratio of 10:90, 20:80, 30 :70,40:60,50:50,60:40,70:30,80:20,90:10. All ratios were subjected to nitric oxide scavenging assay to find out the effective combinational mixture. The ratio 70:30 (*Terminalia belerica* : *Solanum xanthocarpum*) showed maximum free radical scavenging property. The present results suggest that traditional crude drugs might be potent and novel therapeutic agents for scavenging of NO and the regulation of pathological conditions caused by excessive NO and its oxidation product. These findings may also help to explain, at least in part, certain pharmacological activities of crude drugs, especially anti-infection and anti-inflammatory activities.

KEYWORDS: *Solanum Xanthocarpum* (entire plant) , *Terminalia Belerica* (fruit) , Herbal Extract Mixture Preparation. Tannin and Nitric Oxide Free Radical Scavenging Assay, DPPH Method

1. INTRODUCTION:
Herbs are prime medicinal agents, which is used used for its scent, flavor or therapeutic properties. Currently Phytochemistry have significant development. The technology involves the isolation, extraction, purification and characterization of active constituent from natural origin. The isolated lead compounds are mainly used as therapeutic agent in chronic diseases[1].

Keeping this as an idea a well known folklore plants *Solanum xanthocarpum* and *Terminalia belerica* were selected for the preparation of herbal extract mixture.[2] Herbal ingredients used in combination are widely used in Europe, and their assessment is often performed according to specific guidelines. Combinations of herbal and homeopathic ingredients exist in a few countries[3].

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2.MATERIALS AND METHODS:
2.1 Material
The selected plant materials were collected from in and around places of Chennai, Tamilnadu. and authenticated by renowned botanist and its record,voucher specimen,herbarium was maintained and deposited in the Department of Pharmacognosy, School of Pharmaceutical Sciences, VELS University.

2.2 Preparation of extract
The selected plants (500 gm) of the powdered materials were extracted separately by cold maceration procedure successively with solvents of increasing polarity (Petroleum ether (60-80°C), Ethanol and Water).

2.3 Selection of extract
Preparation of herbal extract mixture was mainly based on in vitro antioxidant activity by Nitric oxide scavenging method. Petroleum ether, ethanol and water extract of both the selected plants (*Terminalia belerica*

and *Solanum xanthocarpum*)[4] were screened for free radical scavenging property by Nitric oxide scavenging assay method [5].

2.4 Method

2.4.1 nitric oxide free radical scavenging activity (Harlalka., 2007)

In this assay 1.0 ml of sodium nitroprusside (5mM) in phosphate buffered saline(PBS) was mixed with 1.0 ml of different concentration (25- 900µg/ml) of the herbal extract mixture dissolved in the water. The assay mixture was then incubated at 25°C for 30 minutes. Then this solution was treated with Griess reagent (Sulphilamide- 1%, O- Phosphoric acid- 2%, Napthyl ethylene diamine dihydro chloride – 0.1%). Then the optical density of the resultant chromophore determined spectrophotometrically at 546nm. The results were compared with standard Ascorbic acid.The result of Nitric oxide scavenging assay of *Solanum xanthocarpum* and *Terminalia belerica* are given in Table 1 and 2.

2.4.2 Selection of ratio

The selected bioactive aqueous extracts of both the plants were mixed in different ratios such as 10:90, 20:80, 30:70, 40:60,50:50, 60:40,70:30, 80:20, 90:10 (*Terminalia belerica* : *Solanum anthocarpum*) [6]. All the above prepared herbal mixture were subjected to antioxidant property by in vitro method (Nitric oxide scavenging assay) and % of inhibition was calculated and the reports are tabulated.

2.5 1.1 diphenyl, 2- picryl, hydrazyl (dpph) free radical scavenging assay (Sree Jayan et al., 1997)[7]

DPPH scavenging activity was measured by spectrophotometric method. The methanolic solution of DPPH (0.1mM, 1 ml) was incubated with 3 ml of different concentration of the extract mixture ranging from 25 to 900µg/ml. Incubation was carried out at 25°C for 30 min. At the end of the incubation period, the optical density of each sample was determined at 517nm. The results were compared with standard Ascorbic acid [8].

3. RESULT AND DISCUSSION:

3.1 Extraction

The selected plants were extracted successively by various solvents of increasing polarity (Petroleum ether, Ethanol and Aqueous) by cold maceration process[9]. The percentage yield of the extracts were given in Table 1. Percentage yield of aqueous extract of *Terminalia belerica* and *Solanum xanthocarpum* were found to be 9.9 % w/w and 15.64% w/w.

3.2 Selection of extract[10]

All the extracts of both the plants were subjected to Nitric oxide scavenging assay. The results of various extract are tabulated 2 and 3. Among the tested extracts, aqueous extracts showed maximum percentage of inhibition for both the plants.

3.3 Nitric oxide free radical scavenging activity[11]

The results of in vitro antioxidant assay of various extracts of plant revealed that the aqueous was found to be bioactive potent extract. So aqueous extract of the both plants was selected and prepared in different combination ratio from 10:90, 20:80 30:70, 40:60, 50:50,60:40,70:30,80:20,90:10 (*Terminalia belerica* : *Solanum xanthocarpum*). The results are Tabulated in Table 3.

3.4 DPPH[12]

The selected herbal extract mixture was further subjected to DPPH assay to support to its anti-oxidant activity. Reduction of DPPH radicals can be observed at 517nm. The scavenging activity of herbal extract mixture {*Terminalia belerica* (70): *Solanum xanthocarpum* (30)} was done and Tabulated at

RESULTS:

The selected extract was subjected to *in- vitro* anti oxidant study and their results are tabulated below**.**

Table 1: Nitric oxide scavenging assay of Solanum xanthocarpum

S. No	Extract	Concentraction	Absorbance	% of inhibition
1.	Petroleum ether	Control	0.04	---
		100µg/ml	0.18	6.66
		300 µg/ml	0.18	6.66
		500 µg/ml	0.19	11.76
		700 µg/ml	0.20	17.76
		900 µg/ml	0.21	23.52
2.	Ethanol	Control	0.02	--
		100µg/ml	0.19	5.55
		300 µg/ml	0.20	11.11
		500 µg/ml	0.22	22.22
		700 µg/ml	0.23	27.78
		900 µg/ml	0.24	33.33
3.	Aqueous	Control	0.05	--
		100µg/ml	0.14	7.69
		300 µg/ml	0.15	15.38
		500 µg/ml	0.16	38.46
		700 µg/ml	0.18	46.15
		900 µg/ml	0.20	53.84

From the above result, Aqueous extract of *Solanum xanthocarpum* produced maximum antioxidant activity (53.84%) as compared to the other tested extracts.

Table 2: Nitric oxide scavenging assay of *Terminalia belerica*

S. No	Extract	Concentraction	Absorbance	% of inhibition
1.	Petroleum ether	Control	0.03	--
		100µg/ml	0.17	6.66
		300 µg/ml	0.18	11.76
		500 µg/ml	0.19	17.64
		700 µg/ml	0.20	29.41
		900 µg/ml	0.22	35.29
2.	Ethanol	Control	0.02	--
		100µg/ml	0.19	5.55
		300 µg/ml	0.20	11.11
		500 µg/ml	0.22	22.22
		700 µg/ml	0.23	27.78
		900 µg/ml	0.24	33.33
3.	Aqueous	Control	0.04	--
		100µg/ml	0.16	23.07
		300 µg/ml	0.18	38.46
		500 µg/ml	0.19	46.15
		700 µg/ml	0.20	53.84
		900 µg/ml	0.21	61.53

From the above result, it was found that Aqueous extract of *Terminalia belerica* produced maximum antioxidant activity (61.53%) as compared to the other extract tested. Antioxidant activity results of various extracts of both the plants indicated that aqueous extract of the plants was found to exhibit maximum percentage of inhibition. So, aqueous extract of plants was selected for preparation of herbal extract mixture.

Table 3: Nitric oxide scavenging assay of herbal extract mixtures

S. No	Herbal Extract Ratio	Absorbance					% of inhibition				
	Terminalia : *Solanum*	100 µg/ml	300 µg/ml	500 µg/ml	700 µg/ml	900 µg/ml	100 µg/ml	300 µg/ml	500 µg/ml	700 µg/ml	900 µg/ml
1.	10 : 90	0.14	0.15	0.16	0.16	0.17	7.14	15.38	23.07	23.07	30.76
2.	20 : 80	0.14	0.15	0.16	0.17	0.18	7.14	15.38	23.07	30.76	38.46
3.	30 : 70	0.15	0.16	0.17	0.18	0.19	15.38	23.07	30.76	38.46	46.15
4.	40 : 60	0.14	0.15	0.18	0.19	0.20	7.14	15.38	38.46	46.15	53.84
5.	50 : 50	0.15	0.16	0.17	0.18	0.20	15.38	23.07	30.76	38.46	53.84
6.	60 : 40	0.14	0.16	0.18	0.20	0.20	7.69	23.07	38.46	53.84	61.53
7.	**70 : 30**	0.18	0.21	0.22	0.23	0.24	38.46	61.53	69.23	76.92	**84.61**
8.	80 : 20	0.15	0.17	0.18	0.19	0.20	15.38	30.76	38.46	46.15	53.84
9.	90 : 10	0.15	0.16	0.17	0.18	0.19	15.38	23.07	38.46	38.46	46.15

Among the tested, different ratio of aqueous extract of plants, the ratio **70:30** (aqueous extract of ***Terminalia belerica*** : aqueous extract of ***Solanum xanthocarpum***) was found to produce high percentage of inhibition about **84.61%**. So, this combination herbal extract mixture has more free radical scavenging activity.The selected herbal extract combination was further subjected for its scavenging activity by DPPH method .

Table 4: Free radical scavenging activity of herbal extract mixture by DPPH assay

S. No	Concentration (µg/ml)	% of Inhibition
1.	Control	-
2.	25	08.57±2.36**
3.	50	15.65±3.89**
4.	100	24.33±1.13**
5.	300	42.50±1.16**
6.	500	63.65±1.97**
7.	700	72.20±1.06**
8.	900	81.45±0.74**
9.	Standard (900) (Ascorbic acid)	88.74±0.054

Values are mean ± SEM (n=3); All the values are ** P< 0.01 when compared against control. DPPH

Reduction of DPPH radicals can be observed at 517nm. The scavenging activity of herbal extract mixture { *Terminalia belerica* (70) : *Solanum xanthocarpum* (30)} was found to be 81.45% at 900 µg/ml. Different concentration showed significant P<0.01 activity as compared to control. IC_{50} value of extract mixture was found to be 378µg/ml.

4. DISCUSSION:

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care for its synergetic activity[13]. All the extracts of both the plants were subjected to Nitric oxide scavenging assay. Among the tested extracts, aqueous extracts showed maximum percentage of inhibition for both the plants. Aqueous extract of *Terminalia belerica* showed 61.53 % of scavenging activity. Aqueous extract of *Solanum xanthocarpum* showed 53.84 % of scavenging activity. Since aqueous extract of both the plants has shown maximum free radical scavenging property, it was selected for the

preparation of herbal extract mixture. Aqueous extract of Terminalia belerica and Solanum xanthocarpum have been mixed in the ratio such as 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10.

All the herbal mixture ratio were screened for free radical scavenging property by nitric oxide assay. Results are given in Table7. Among the tested different combination, the ratio 70:30 (Terminalia belerica: Solanum xanthocarpum) exhibited significant free radical scavenging property (84.61%). The free radical scavenging property of herbal extract mixture was tested at different concentration and results are shown in Table 8 and figure 13. All tested concentrations of extract mixture showed significant $P<0.01$ activity as compared to control. IC_{50} value of extract mixture was found to be 200µg/ml. Reduction of DPPH radicals can be observed at 517nm. The scavenging activity of herbal extract mixture {Terminalia belerica (70): Solanum xanthocarpum (30)} was found to be 81.45% at 900 µg/ml. Different concentration showed significant $P<0.01$ activity as compared to control. Results are shown in Table 9 and figure 14. IC_{50} value of extract mixture was found to be 378µg/ml.

5. CONCLUSION:

In this pilot study much effort has been directed at proving the benefits of antioxidants, the findings to date are far from clear[14]. Herbal extract mixture was prepared in different combination (aqueous extract of *Terminalia belerica* : aqueous extract of *Solanum xanthocarpum*). The ratio 70:30 (*Terminalia belerica*: *Solanum xanthocarpum*) showed maximum free radical scavenging property. On the basis of above results, it can be concluded that the antioxidant potential of the plant extract depends on the presence of phenolic compounds and tannins. There is however a scope for confirming the results of this study on relevant animal models followed by studies for clinical support in future for development of herbal formulation of with respective plant extracts.

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