In-Vitro Evaluation of Antioxidant Activity of Five Drugs of *Trinpanchmool*

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Summary

In Ayurveda, group of drugs known as "Trinpanchmool" includes Kush (*Desmostachya bipinnata)*, Kas (*Saccharum spontaneum)*, Darbh (*Imperata cylindrica)*, Sar (*Saccharum munja)* and Ikshu (*Saccharum officinarum)* of Poaceae family. In Ayurveda, Trinpanchmool drugs are prescribed for the treatment of various ailments like asthma, jaundice, galactogogue, diuretic, gout, leprosy, anemia, eye disease and also used as aphrodisiac. Since present, there is no research reporting antioxidant claim of Trinpanchmool. The aim of this study was to determine the antioxidant activity of 5 different drugs of Trinpanchmool in separate and in combination form. For this purpose, root of Kush, Kas, Darbh, Sar and Ikshu were procured and identified. Individually all these drugs and in combination form were subjected for hydroalcoholic extraction. The antioxidant activity of individual as well as the combination of all five was tested by adopting DPPH method and Ferric reducing antioxidant power assay method. In these tests, the combinations of all five drugs shown better antioxidant activity in terms of % inhibition and IC_{50} in comparison to individual drugs.

Key Words: Trinpanchmool; Antioxidant; Free radical scavenging activity DPPH; FRAP.

Introduction

Trinpanchmool is a group of five Ayurvedic drugs namely Kush (*Desmostachya bipinnata)*, Kas (*Saccharum spontaneum)*, Darbh (*Imperata cylindrica)*, Sar (*Saccharum munja)* and Ikshu (*Saccharum officinarum)* of Poaceae family [1,2]. These are distributed in hotter and dry parts of the country. Root stock of Kush mainly contains terpene and used in disorders like asthma and jaundice. Root of Darbh contains five triterpenoid viz. cylindrin, arundoin, ferneon, isobarborneol, cimilarenol and is used in urinary calculi, retention of urine, diabetes, cardiac disorder, gout and inflammation, aphrodisiac. Plant of Kas contains protein, calcium phosphate, hydrocyanic acid glycosides and is used as a galactogogue and diuretic. Sar stem is sweet acrid cooling, aphrodisiac useful in burning sensation thirst, erysipelas blood troubles, urinary complains, eye disease and is a good source of furfural. Stem and root of Ikshu contains sucrose, glucose, fructose, aspargin and glutamine prominent amino acids in juice and useful in fatigue thirst, leprosy, anemia, erysipelas. All five drugs are used individually as well as in combination in traditional system of medicine [1].

Free radicals are continuously produced by the body's normal use of oxygen such as respiration and some cell mediated immune functions [3]. Free radicals attack the nearest stable molecule, taking its electron when the 'attacked' molecule loses its electron; it becomes a free radical itself, beginning a chain reaction[4]. Once the process is started, it can continue, finally resulting in the disruption of a living cell [5]. Naturally there is a dynamic balance between the amount of free radicals generated in the body and antioxidants to quench and/or scavenge them and protect the body against their deleterious effects [6-8]. The cause of a majority of disease conditions like atherosclerosis [9], hypertension [10], ischaemic diseases [11], alzhemiers diaease[12], parkinsonism [13], cancer [14], diabetes mellitus [15] and inflammation conditions [16] are being considered to be primarily due to the imbalance between oxidant and antioxidant homeostasis. There are many herbs, herbal drugs, synthetic drugs and food supplements are available for reestablishments of this balance [17] but the present scenario is showing the replacement of synthetic agents with herbals in nearly all filed of medicine [18]. In Ayurvedic system of medicine, Trinpachmool is one among those formulations. So in this present study, the antioxidant activity of all five drugs indivisually and in combination was evaluated.

Experimental

Plant material collection and authentication

Roots of all the five drugs naming Kush (*Desmostachya bipinnata)*, Kas (*Saccharum spontaneum)*, Darbh (*Imperata cylindrica)*, Sar (*Saccharum munja)* and Ikshu (*Saccharum officinarum)* were collected from Banaras Hindu University campus, Varanasi and authenticated by Dr. V. K. Joshi, Dean of Faculty of Ayurveda, Institute of Medical Science, B.H.U., Varanasi and also through National Botanical Research Institute (NBRI), Lucknow. A Voucher specimen of all the plants has been preserved in the Department of Pharmacognosy, College of Pharmacy, IFTM, Moradabad for further references.

Extraction

The roots were separated, washed, dried under shade and then grinded and coarsely powdered. The powdered material of all the drugs was subjected to hydroalcoholic extraction in Soxhlet apparatus. The solvent was then removed under reduced pressure and used for evaluation for their antioxidant activity by following methods.

DPPH radical scavenging activity

DPPH is a common abbreviation for an organic chemical compound 2,2-diphenyl-1-picrylhydrazyl[19]. It is a dark-colored crystalline powder composed of stable free-radical molecules. The DPPH assay measures the hydrogen atom (or one electron) donating activity DPPH, which was reduced into the diphenylpicryl hydrazine (a yellow colored compound) which is measured spectrophotometrically at 517 nm[20]. Stock solutions of all five extracts and combination of them were prepared (1.0 mg/ml). These sample stock solutions were diluted to final concentrations of 50, 40, 30, 20, 10 μg/ml, in ethanol. One ml of a 0.3 mM DPPH ethanolic solution was added to 2.5 ml of sample solutions of different concentrations and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 518 nm and converted into percentage of antioxidant activity (AA) using the following formula:

$$\mathbf{AA\ \% = 100 - \{[(ABS_{SAMPLE} - ABS_{BLANK})\ X\ 100] / ABS_{CONTROL}\}}$$

DPPH solution (1.0 ml; 0.3 mM) plus ethanol (2.5 ml) was used for blank. For standard, ascorbic acid was used. Tests were performed in triplicate and average was calculated.
Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. ED_{50} values of the scavenging assay (concentration which can achieve 50% scavenging) were calculated by plotting the percentage of inhibition against the concentration to quantify the activity.

Evaluation of antioxidant activity using FRAP method
The principle of this method is the reduction of a ferric-tripyridyl triazine complex to its colored ferrous form in the presence of antioxidants[21]. FRAP agent was prepared by mixing 25 ml of acetate buffer (500 mM/l) with 2.5 ml of tripyridyltriazine (TPTZ) (10 mM/l) and 2.5ml of ferric chloride (20 mM/l) solution[22]. The reaction mixture contained 300 µl of freshly prepared FRAP reagent warmed to 37^{o} C, added to 50, 40, 30, 20, 10 µg/ml concentrations of all extracts along with 30 µl of water. Absorbance of these solutions were taken at 593 nm, just after 4 min from the time of addition of FRAP reagent. An increase in absorbance indicated enhanced reducing potential of extracts. Quantitative calculation for each sample was done by plotting calibration curve and line equation. Aqueous solution of known Fe (II) concentration was used for calibration. The equation used for Absorbance was as (y) = 0.274 x + 0.114 [r^2 = 0.974]
The antioxidant activities were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mM/l FeSO4.
Percentage inhibition= [1− (absorbance of test/absorbance of control)] ×100

Statistical analysis
The values were reported as mean ± SD. One-way ANOVA and Dunnet comparison tests were used for the statistical analysis.

Results and Discussion

In the present study, free radical scavenging activities of all five drugs and in combinations were measured by DPPH and FRAP assay method and all results were compared with standard antioxidant (Ascorbic acid).

DPPH radical scavenging activity
Test solutions of concentration 10-50µg/ml were prepared for every individual drug and for the combination. Absorbance was checked and % inhibition was calculated by the formula. Results of the DPPH test are presented in table 1.
Antioxidant plant extract reacts with DPPH, which is a nitrogen-centered radical with a characteristic absorption at 517 nm and is being converted into 1, 1-diphenyl-2-picryl hydrazine, due to its hydrogen binding ability at a very fast rate [23]. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical proceeds, which results in the scavenging of the radical by hydrogen donation. Table 1 illustrates increase scavenging of DPPH radicals in dose dependent manner due to the scavenging ability and the combination extract of all five drugs was found to be more potent antioxidant than all other individual drugs. IC_{50} value of ascorbic acid is 6.351±1.01 µg/ml.

Table 1. % Inhibition by DPPH method

Conc. (µg/ml)	Percentage Inhibition						
	Kush[a]	Kas[a]	Darbh[a]	Sar(Munja)[a]	Ikshu[a]	Combination[a,b]	Standard
10	9.20±0.038	7.23±0.066	14.33±0.045	12.23±0.059	13.93±0.080	20.51±0.085	74.27±0.052
20	15.90±0.081	18.94±0.063	19.26±0.076	21.00±0.086	21.87±0.076	27.74±0.074	77.30±0.068
30	20.60±0.091	26.79±0.080	28.44±0.023	33.55±0.060	31.87±0.081	36.51±0.022	81.54±0.074
40	25.24±0.035	28.99±0.062	30.93±0.066	37.37±0.040	34.93±0.038	39.08±0.049	84.90±0.038
50	36.46±0.073	36.11±0.060	36.56±0.053	40. 47±0.121	36.91±0.093	45.33±0.102	89.91±0.100

[a]Data expressed as mean ± SD. Each sample was analyzed in triplicate.
[b]A combination of all five drugs.

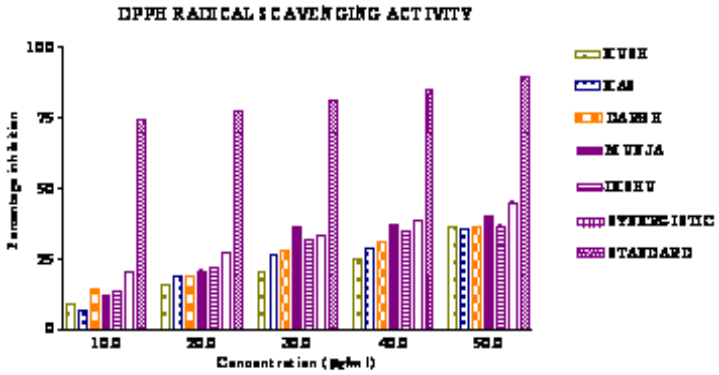


Fig. 1 DPPH radical scavenging activity of all five drugs, combination extract and standard

Assay by FRAP method

In this method, measurement of the ferric reducing ability (FRAP) is done. At low pH, when a ferric- Tro-tripyridyl triazine (FeIII-TPTZ) complex is reduced to ferrous (FeII) form, an intense blue color with an absorption maximum at 593 nm develops. Test conditions favor reduction of the complex and thus the color development indicates that an extract is having antioxidant potential. For the measurement of the antioxidant activity, the $Fe^{+3} \rightarrow Fe^{+2}$ conversion in the presence of all six hydroalcoholic extracts were observed and it was found that the combination of all five drugs shown better free radical scavenging activity in comparison to all individual drugs. For FRAP method, ascorbic acid was used as standard. The calibration curve was drawn for Fe^{++}. The line equation was found to be y = 0.274 x + 0.114 [r^2 = 0.974]. The result of antioxidant activity of all five extracts, combination extract and standard are presented in table 2.

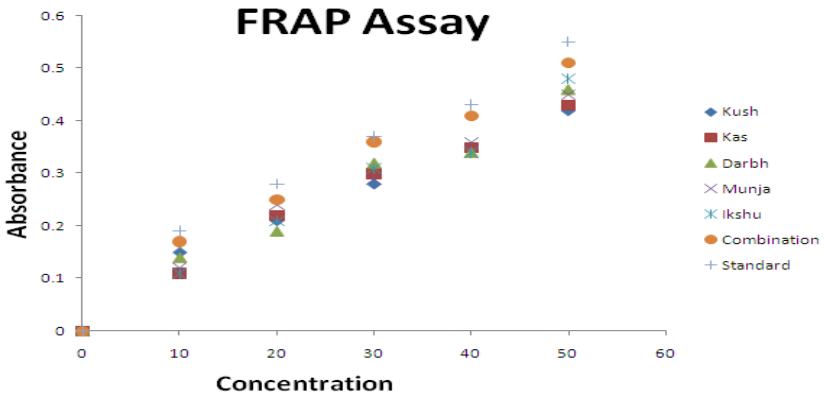
Table 2: FRAP assay hydroalcoholic extracts of all five drugs extracts, combination extract and standard

Conc. (µg/ml)	Absorbance						
	Kush	Kas	Darbh	Sar(munja)	Ikshu	Combination	Standard
10	0.15	0.11	0.14	0.12	0.11	0.17	0.19
20	0.21	0.22	0.19	0.24	0.21	0.25	0.28
30	0.28	0.30	0.32	0.29	0.31	0.36	0.37
40	0.35	0.35	0.34	0.36	0.34	0.41	0.43
50	0.42	0.43	0.46	0.45	0.48	0.51	0.55

Table 3: IC50 values of test performed by DPPH and FRAP assay method

Drug	DPPH method*	FRAP assay*
Kush	78.04	23.69
Kas	58.45	12.87
Darbh	53.11	18.86
Munjha	57.44	13.81
Ikshu	63.47	19.65
Combination	51.14	11.31

*The results are presented in average of three readings.



The free radical scavenging activity by FRAP method was found to be concentration dependent and in the combination extract, activity was found to be significant different from all other individual drugs generally at all concentration tested. From absorbance measured, %inhibition was measured and IC_{50} values were calculated. The results are presented in table 3.

Conclusion

The antioxidant action of reductants is based on the breaking of the free radical chain by donating a hydrogen atom. Significant differences ($P < 0.001$) in antioxidant potential of all individual drugs when compared with combination extract and standard was found and it was concluded that though the five drugs Kush, Kas, Darbh, Sar, Ikshu hydroalcoholic extract shown significant antioxidant activity when tested by DPPH and FRAP method but the combination extract shown more prominent free radical scavenging activity in comparison to individual drugs. The IC_{50} of the combination extract by DPPH and FRAP method were found to be 51.15 and 11.31 respectively. This was indicative that the combination extract is having significant free radical scavenging potential than the individual drugs. The results of this research suggested that combination of all five drugs (Trinpanchmool) may be used as potential remedy to treat several ailments which are associated with free radicals.

References

[1] Anonymous (2000). The Ayurvedic Formulary of India, Part (II), Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homeopathy(pp 70-72), Govt of India, New Delhi .

[2] Sharma, P. C., Yelne, M. B. and Dennis, T. J.(2002). Database on Medicinal Plants Used in Ayurveda, (pp187-190)CCRAS, Department of Indian System of Medicine and Homeopathy, Govt. of India, New Delhi.

[3] Lata, H., and Ahuja, G. K. Role of free radicals in health and disease. Indian Journal of Physiology and Allied Science. (2003) 57, 124-130

[4] Galvez, M., Martin-Cordero, C., Houghton, P. J., and Ayuso, M. J. Antioxidant activity of methanol extracts obtained from Plantago species. Journal of Agriculture and Food Chemistry. (2005) 53, 1927-1933

[5] Schuler, P. (1990). Natural antioxidants exploited commercially. In: Hudson BJF. (ed.) Food Antioxidants. (pp 99-107). Elsevier, London. 99-170.

[6] Ahmed, R. G. (2005). Is there a balance between oxidative Stress and antioxidant defense system during development? Medical Journal of Islamic World Academy of Science 15(2), 55-63.

[7] Toyokuni, S. (1999). Reactive oxygen species-induced molecular damage and its application in pathology. Pathology International. 49, 91-102.

[8] Zimmerman, J. J. (1998). Redox/radical repertoire rapport: Pathophysiology and therapeutics. Acta Anaesthesiology Scand. 42, 1-3.

[9] Niki, E. (2004). Antioxidants and atherosclerosis. Biochemical Society Transactions. 32(1), 156-159.

[10] Nwanjo, H. U., Oze, G., Okafor, M. C., Nwosu, D., and Nwankpa, P. (2007). Oxidative stress and non-enzymic antioxidant status in hypertensive patients in Nigeria. African Journal of Biotechnology. 6 (14), 1681-1684.

[11] Bansilal, Ali, N., Afzal, N., Khan, T. S., and Shahjahan, S. (2007). Antioxidant status in coronary heart disease (CHD) patients with type 2 diabetes mellitus. Journal of Ayurvedic Medical College Abbottabad .19(4), 98-101.

[12] Frank, B., and Gupta, S. (2005). A review of antioxidants and Alzheimer's disease. Annals of Clinical Psychiatry. 17(4), 269-86.

[13] Ebadi, M., Srinivasan, S. K., and Baxi, M. D., (1996). Oxidative stress and antioxidant therapy in Parkinson's disease. Progress in Neurobiology. 48(1), 1-19.

[14] Jaruga, P., and Oliński, R. Activity of antioxidant enzymes in cancer diseases. Postepy Higieny Medycyny Dosw. (1994) 48(4), 443-55.

[15] Dickinson, P. J., Carrington, A. L., Frost, G. S., and Boulton, A. J.M. (2002). Neurovascular disease, antioxidants and glycation in diabetes. Diabetes/Metabolism Research and Reviews. 18(4), 260–272

[16] Marian, V., Dieter, L., Moncola, J., Mark, T. D. C., Mazura, M. and Joshua, T. Free radicals and antioxidants in normal physiologicalnfunctions and human disease. The Intern. J. Biochem. & Cell Biol. doi:10.1016/j.biocel.(2006).07.001.

[17] Ashok ,D. B., and Thomas P.A. (2007). Current Status of Herbal Drugs in India: An Overview. Journal of Clinical Biochemistry and Nutrition 41(1): 1–11.
[18] Rai, J. (2005). Popularity of Herbal Drugs -A Challenge to Rational Practice. Herbal Insights 7(3), 180-182.
[19] Kai, M., Klaus, H. V., Sebastian, L., Ralf, H., Andreas, R., and Ulf-Peter, H. Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of Spectrophotometric measurements. Sensors (2007) 7, 2080-2095.

[20] Yuan, Y., Chen, C., Yang, B., Kusu, F. and Kotani, A. (2009). DPPH radical scavenging activities of 31 flavonoids and phenolic acids and 10 extracts of Chinese materia medica. Zhongguo Zhong Yao. Za. Zhi. 34(13), 1695-1700.

[21] Benzie, I. F., and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry. 15, 70-76.

[22] Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. and Hawkins, B. D. (2006) .Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis. 19, 669–675
[23] Yamaguchi, T., Takamura, H., Matoba, T. and Terao, J. (1998). HPLC method for evaluation of the free radical scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. Bioscience, Biotechnology and Biochemistry. 62, 1201-1204.