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Original Article

ASSESSMENT OF FREE RADICAL SCAVENGING ACTIVITY OF TEN MADHURASKANDHA DRUGS THROUGH UV SPECTROSCOPIC AND CHROMATOGRAPHIC TECHNIQUE

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ABSTRACT

Objective: Acharya Charaka had classified *Asthapana bastidravya* (corrective enema) based on Rasa (Taste), called as *Rasaskandha* (a group of drugs having similar taste). He ascertained some criteria to include drugs in the group such as drug having either similar *Rasa* (taste) or *Vipaka* (biotransformation) or *Prabhava* (principle responsible for a specific action). The study was planned to endorse the grouping of Madhuraskandha based upon *Rasayana* karma (rejuvenation) vis a vis antioxidant activity as well as to evaluate the natural source of antioxidants.

Methods: The study was planned to assess the free radical scavenging activities often drugs by adopting DPPH [2, 2-di-(4-tert-octylphenol)-1-picrylhydrazyl], H_2O_2 (Hydrogen peroxide) and superoxide scavenging activity and FRAP (Ferric reducing antioxidant power) assay with UV spectroscopy. Further, methanolic extract often drugs was subjected to thin layer chromatography followed by DPPH technique.

Results: The aqueous extracts of *Abutilon indicum, Solanum xanthocarpum, Tribulus terrestris, Boerhavia diffusa,* Group A and Group C had shown potent antioxidant activity in concentration-dependent manner by illustrated methods. DPPH-TLC (thin layer chromatography) assay showed that maximum active compounds were found in methanolic extracts of *Asparagus racemosus* and *Tinospora cordifolia*.

Conclusion: The result revealed that the drugs included in the Madhuraskandha have similar attributes of *Rasayana Karma* or possess antioxidant activity. The combination of ten drugs can be a good source of natural antioxidants.

Keywords: Madhuraskandha drugs, Free radical scavenging activity, Spectroscopy, Chromatography

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INTRODUCTION

Classification allows structuring the thematic communication message. In Ayurveda, dravyas were classified on the basis of *Chetana-Achetana* (activity), *Karya-karana* (cause and effect), *Utpatti* (evolution), *Yoni* (Source), *Prayoga* (utility), *Rasa* (Taste), *Veerya* (Potency) etc. Acharya Charaka had classified *Asthapanabasti* dravyas based on the *Rasa*. He stated that the drugs included in each category do possess similar Rasa or Rasa oriented Vipaka or *Prabhava* e. g. the drugs enlisted in the *Madhuraskandha* have *Madhurarasa* or *Madhuravipaka* or *Madhuraprabhava* (drugs are not having *madhurarasa* or *madhuravipaka* but show the actions like *Madhurarasa* and *vipaka*) [1]. *Madhurarasa* is attributed for *Balya* (strengthening), *Brihmaneeya* (increasing muscle bulk of the body), *Jeevaniya* (invigorating), *Rasayana* (rejuvenating) Karmas while *Madhuravipaka* has *Shukrala* (spermatopoeitic) property [2].

According to modern pharmacology, oxidation is a chemical reaction involving the loss of electrons or an increase in oxidation state. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chains of reactions by removing free radical intermediates and inhibit other oxidation reactions. Likewise, *Rasayana* drugs either act through *Deepana, Pachana* karma at the metabolic level. They increase *Dhatvagni* (the enzyme responsible for the metabolism of each *dhatu*/tissue) or directly nourishing the *Dhatus (tissues) with the help of Guru (heavy to digest), Snigdha (unctuous), Sheeta (cold)* by *Balya*, *Brihmaniya*, *Jeevaniya* karma. So that they can protect the cell and promote the cell longevity as that of antioxidants.

Nowadays, synthetic and natural antioxidants are routinely used in foods and medicine especially those containing oils and fats to protect against oxidation. There are a number of synthetic antioxidants like Butylated hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA). However, some physical properties as high volatility and instability at elevated temperature, and its carcinogenic nature [3] turned the consumer's preference towards natural sources of antioxidants, which are cost effective, highly compatible with dietary intake and minimal side effect to human body [4].

Keeping this in view to validate the grouping of the drugs in the Madhuraskandha on the basis of *Rasayana Karma* vis a vis antioxidant activity and to find out the natural source of antioxidants the ten drugs of Madhuraskandha were studied for their free radical scavenging activity by adopting different *in vitro* methods.

Table 1: Protocol for collection of the Madhuraskandha drugs

S. No.	Individual drug	Botanical Source	Part used	Time of collection	Place of collection
1.	*Atibala*	*Abutilon indicum* Linn. Sweet	Root	May 2014	Periphery of Jamnagar
2.	*Vidari*	*Pueraria tuberosa* DC.	Tuber	Nov 2014	Junagadh
3.	*Kantakari*	*Solanum virginianum Linn.*	Whole plant	July 2014	Periphery of Jamnagar
4.	*Eranda*	*Ricinus communis* Linn	Root	May 2014	Periphery of Jamnagar
5.	*Gokshura*	*Tribulus terrestris* Linn	Fruit	Oct 2014	Periphery of Jamnagar
6.	*Guduchi*	*Tinospora cordifolia* (Willd.) Miers ex Hook. f. &Thoms	Stem	Sept 2014	Periphery of Jamnagar
7.	*Shalaparni*	*Desmodium gangeticum* DC.	Root	July 2014	Junagadh
8.	*Jivanti*	*Leptadenia reticulata* W. & A.	Leaves	July 2014	Periphery of Jamnagar
9.	*Shatavari*	*Asparagus racemosus* Willd.	Tuberous root	Jun 2014	Periphery of Jamnagar
10.	*Punarnava*	*Boerhavia diffusa* Linn	Root	July 2014	Periphery of Jamnagar

MATERIAL AND METHODS

Collection of drugs

The drugs were collected in their respective season according to the part used. The details are mentioned below (table 1). The collected drugs were washed, cleaned and dried in the shade. The dried drugs were powdered individually and sieved through mesh 85. The drug samples were authenticated through powder microscopy, preliminary physicochemical and phytochemicals procedures by following the standard procedure mentioned in Ayurvedic pharmacopeia of India (API) in the Laboratory of Pharmacognosy and Laboratory of Pharmaceutical chemistry, I. P. G. T. & R. A, Jamnagar [5].

Criteria for grouping

The selected ten drugs, as well as their three combinations, were evaluated for *in vitro* antioxidant activity. Considering the evaluation of *Rasayana* (antioxidant) activity, the drugs were grouped by comparing and analyzing with *Vayasthapana dashemani* group (Group of ten drugs having anti-aging property). The drugs which are easily available and found to be uncommon to both the group (*Madhuraskandha* and *Vayasthapana dashemani*) were included in Group A and common drugs were incorporated in Group B. Group C was the combination of Group A and Group B.

Preparation of extract

About 5g of the test drug powders were macerated with distilled water (100 ml) in a closed flask for 24 h with frequent shaking for first 6 h and kept as it is for 18 h. After 24 h it was filtered, and the filtrate was taken into a petri dish and evaporated at low temperature using a water bath. The percentage of the extract was calculated by using Eq. (1). The same procedure was followed for methanolic extract [6].

% of water extract: [(Weight of Petri dish+dry extract)]-[Weight of petri dish]/Weight of sample ×100 … Eq. (1)

Sample preparation

The dry extract was reconstituted into liquid form by using distilled water and used for the experiment.

Instrument and reagent

Hydrogen peroxide, Di-hydrogen phosphate, Sodium acetate, Glacial acetic acid, HCl, Ferric chloride, ferrous sulfate and NaOH were obtained from Finar Ltd, Mumbai. 1, 1-diphenyl, 1, 1-picryl hydrazyl (DPPH), EDTA; 2, 4, 6-tri [2-pyridyl]-s-triazine (TPTZ) were procured from Sigma, Bangalore. Nitro blue tetrazolium (NBT) was obtained from Hi-Media Pvt. Ltd, Mumbai. Methanol, Toluene, ethyl acetate, formic acid was obtained from Merck specialties Pvt. (Ltd) Mumbai. UV spectrophotometer (UV-VIS double beam spectro-photometer, Shimadzu) was used to find out absorbance of the sample.

Chromatographic conditions

1. Application mode: Camag Linomat V
2. Development Chamber: Camag Twin trough Chamber
3. Plates: Precoated Silica Gel GF254 Plates
4. Chamber Saturation: 30 min
5. Development Time: 30 min
6. Development distance: 8 cm
7. Scanner: Camag Scanner III
8. Detection: Deuterium lamp, Tungsten Lamp
9. Data System: Win cats software
10. Mobile Phase: Toluene: Ethyl acetate: Formic acid (7:2:0.5 v/v)
11. Spray reagent: 0.2 % DPPH solution

Analysis of antioxidant activity through chromatography

The radical containing reagent DPPH is used to detect anti-oxidant properties. DPPH is a stable radical with purple color. The compound turns yellow when reduced by a radical scavenger. The dried plate was sprayed with a solution of 25 mg DPPH in 50 ml methanol (0.2% solution). Any radical scavenging activity was examined when blue radical turned to yellow at least 30s after spraying [7].

Hydrogen peroxide scavenging capacity

The ability of plant extracts to scavenge hydrogen peroxide was determined by following method [8]. Accordingly, a solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). Three different concentration of Extract (25 µg/ml, 50µg/ml and 75µg/ml) prepared in distilled water were added to a 0.6 ml hydrogen peroxide solution. The absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging capacity was calculated by Eq. (2):

$$\% \text{ Scavenged } [H_2O_2] = [(A_C - A_S)/A_C] \times 100 \quad \ldots \text{Eq. (2)}$$

Where A_C is the absorbance of control, A_S is absorbance of sample

DPPH scavenging capacity

Antiradical activity was measured by observing decrease in the absorbance at 517 nm of a Methanolic solution of colored DPPH upon sample treatment [9]. 1.303 mg/ml stock solution of DPPH was prepared such that 75µl of it in 3 ml methanol gave the initial absorbance of 0.9. Decrease in the absorbance in the presence of the test sample of different concentrations was measured at 517 nm up to 2 min with 30 sec intervals. The percentage of the DPPH radical scavenging is calculated using the Eq. (3):

$$\% \text{ inhibition of DPPH radical} = ([A_{br} - A_{ar}]/A_{br}) \times 100 \quad \ldots \text{Eq. (3)}$$

Where, A_{br} is the absorbance before reaction and A_{ar} is the absorbance after the reaction has been noted.

Superoxide scavenging capacity

Superoxide anion radical scavenging assay is based on the reduction of nitroblue tetrazolium to a blue colored formazan in the presence of riboflavin-light-NBT which is directly proportional to the concentration of superoxide anion in the system [10]. The reaction mixture was prepared by adding 200µl EDTA (12 mM), 300µl NBT (1%), 200µl Riboflavin and 3 ml phosphate buffer (50 mM, 7.4pH). The concentration of extracts in the reaction mixture was 10, 20, 30 µg/ml. The solution was mixed well by vortexing. The reaction was initiated by illuminating the sample cuvette with a fluorescent lamp (20W) and measured the increase in absorbance at 590 nm at an interval of 30 sec up to 2.5 min and final measurement at 4 min. Ascorbic acid was used as reference standard. The superoxide radical scavenging activity was calculated using the Eq. (4) below:

$$\% \text{ Inhibition} = [(A_C - A_S)/A_C] \times 100 \quad \ldots \text{Eq. (4)}$$

Where A_C is the absorbance of control, A_S is absorbance of sample

Ferric reducing antioxidant power assay

FRAP assay was performed according to the method mentioned as follow [11]. FRAP reagent was prepared freshly by using 200 ml of acetate buffer, 20 ml TPTZ solution, 20 ml $FeCl_3$ solution and 24 ml distilled water. A blank sample was run by adding 30 µl of distilled water and 1 ml of FRAP reagent to the cuvette and the contents were mixed gently. Initial absorbance was recorded at 593 nm, at 37 °C, and final absorbance was measured after 4 min. Series of stock solution at 0.2, 0.4, 0.6, 0.8, 1.0 mM were prepared (r^2= 0.9129) using aqueous solution of $FeSO_4.7H_2O$ for standard curve. The values obtained were expressed as mM of ferrous equivalent per liter.

Each drug was subjected for assessment of free radical scavenging activity by two methods. Antioxidant activity of the test drugs already reported by certain methods has been taken into consideration and those drugs were evaluated with other available methods to avoid repetition. The allotment of methods is described as follows (Table 2):

Table 2: The distribution of *in vitro* antioxidant methods for Madhuraskandha drugs

S. No.	Anti-oxidant activity	Sample	Code
1.	Hydrogen peroxide scavenging capacity	*Abutilon indicum*	(S1)
		Solanum virginianum	(S3)
		Ricinus communis	(S4)
		Tinospora cordifolia	(S6)
		Asparagus racemosus	(S9)
		Boerhavia diffusa	(S10)
2.	DPPH scavenging capacity	*Pueraria tuberosa*	(S2)
		Tribulus terrestris	(S5)
		Leptadenia reticulata	(S8)
		Group A, Group B and Group C	
3.	Superoxide scavenging capacity	*Abutilon indicum*	(S1)
		Desmodium gangeticum	(S7)
4.	Ferric reducing antioxidant power (FRAP) assay	*Pueraria tuberosa*	(S2)
		Solanum virginianum	(S3)
		Ricinus communis	(S4)
		Tinospora cordifolia	(S6)
		Tribulus terrestris	(S5)
		Desmodium gangeticum	(S7)
		Leptadenia reticulata	(S8)
		Asparagus racemosus	(S9)
		Boerhavia diffusa	(S10)

[Group A-is combination of S1+S2+S3+S4+S5; Group B–S6+S7+S8+S9+S10; Group C-Group A+Group B]

Calculation of IC_{50} value

Three concentrations for each sample were used for the study and IC_{50} values correspond to the concentration of anti-oxidant that leads to a loss of 50% of radical absorbance. It is calculated by plotting graph of concentration vs. absorbance and 50% inhibition was calculated by using regression in MS excel 2010.

RESULTS

Qualitative DPPH assay on TLC

When dried plate was sprayed with DPPH solution, it showed color change from blue to yellow spot after 30 sec. The color change observed at the Rf values mentioned below. These Rf values indicate the number of chemical constituents responsible for the radical scavenging antioxidant activity. The maximum number of compounds was found in S9 responsible for antioxidant activity (table 3).

Hydrogen peroxide scavenging capacity

All the test samples scavenged hydrogen peroxide radical in a concentration dependent manner. The data (fig. 1. [A, B]) Showed that sample S3, S10 and S1 showed significant antioxidant activity than other samples. The free radical scavenging activity decreased in the following order: S3, S10, S1, S9, S6 and S4.

Table 3: Rf values after spraying of DPPH

S. No.	Sample	Botanical name	Rf value	Characteristics of active spot
1.	S1	*Abutilon indicum* Linn. Sweet	0.25,0.43	*
2.	S2	*Pueraria tuberosa* DC.	0.06	-
3.	S3	*Solanum virginianum Linn.*	0.25	*
4.	S4	*Ricinus communis* Linn	0.16,0.23	*
5.	S5	*Tribulus terrestris* Linn	0.06	-
6.	S6	*Tinospora cordifolia* (Willd.) Miers ex Hook. f. &Thoms	0.18,0.3,0.42	**
7.	S7	*Desmodium gangeticum* DC.	0.21, 0.27	*
8.	S8	*Leptadenia reticulata* W. & A.	0.22, 0.32, 0.61	-
9.	S9	*Asparagus racemosus* Willd.	0.21,0.30,0.50,0.61,0.68	**
10.	S10	*Boerhavia diffusa* Linn	0.18	*
11.	Gr1	S1-S5 Samples	0.17	*
12.	Gr2	S6-S10 Samples	0.18, 0.22	*
13.	Gr3	S1-S10 samples	0.18, 0.20	*

[Rf value: retention factor value, *-comparatively weak activity, **-comparatively strong activity]

DPPH scavenging capacity

The reduction capability of DPPH radical was determined by the decrease in absorbance at 517 nm, as a result of antioxidants. Hence, DPPH is often used as a substrate to evaluate antioxidant activity of test compounds. All the samples were scavenged free radical in a concentration-dependent manner (fig. 1[D]).

Among all the test samples, Group C (combination often drugs) had shown significant antioxidant activity with 2.85 mg/ml IC_{50} value when compared with reference ascorbic acid (0.02 mg/ml). The order of sample can be arranged as Group A, S5, Group B, S2 and S8 respectively. The IC_{50} values of all the samples were shown in the fig. 1[C].

Superoxide scavenging capacity

The superoxide radical scavenging activity is significant in S1 as compared to standard ascorbic acid. The IC_{50} value of the samples is given in the fig. 1[E,F].

Ferric reducing antioxidant power assay

The linearity of FRAP (dose–response curve) for standard solutions is shown in fig. 1[G]. The FRAP values were calculated by using linear equation. The FRAP values were found to be increased in concentration dependent manner in S2, S3, S4, S5, S6, S9 and S10 samples except S7 and S8 samples (fig. 1[H]). The antioxidant activities were expressed as the concentrations of antioxidant having a ferric reducing ability equivalent to that of 1 mM of $FeSO_4$.

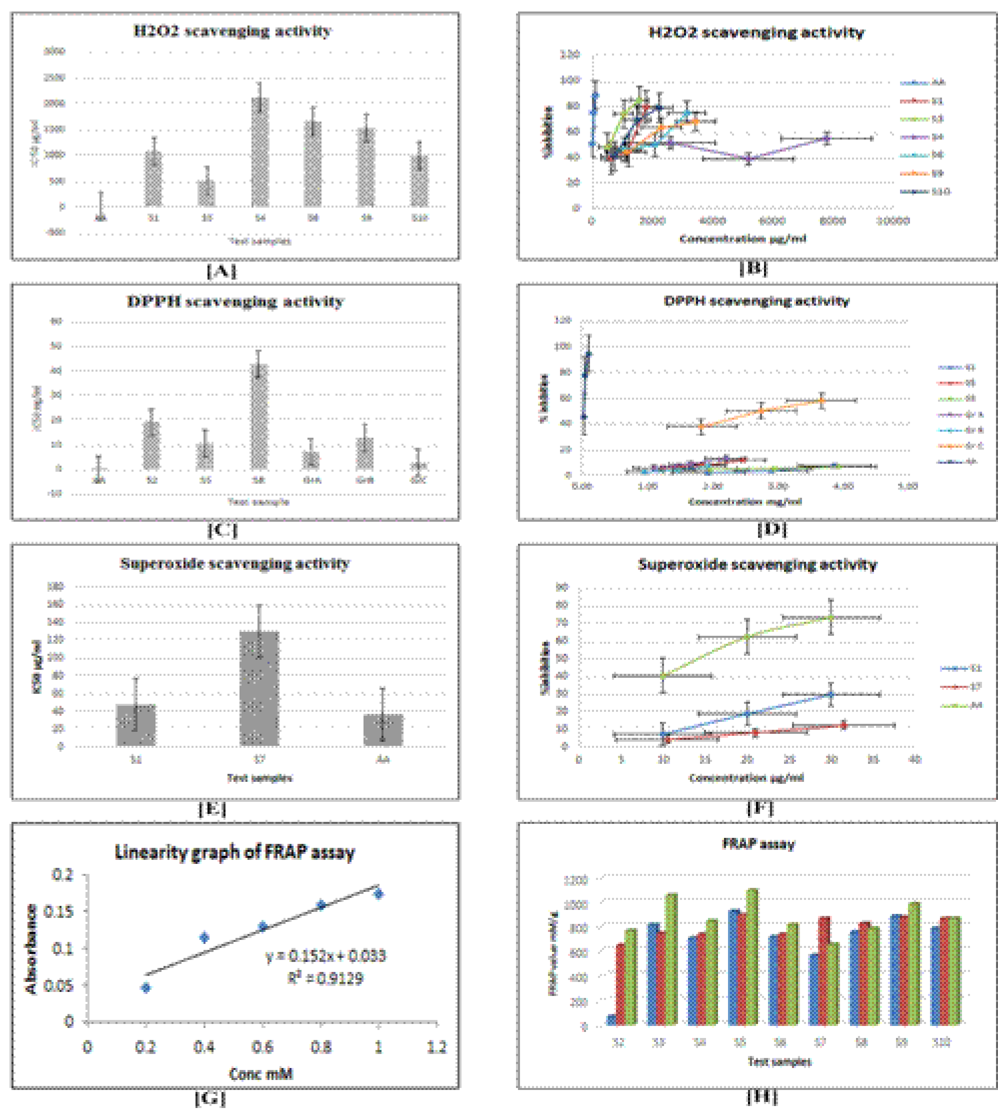

Fig. 1: ***In vitro*** **antioxidant activity of Madhuraskandha drugs**

Fig 1:[A, B] Comparison of H_2O_2 scavenging activity of six test samples [C, D] Comparison of DPPH scavenging activity of six test samples [E, F] comparison of Superoxide radical scavenging activity of two test samples. [G] Linearity of FRAP (dose response curve) of standard solution [H] comparison of FRAP assay of nine test samples. AA- Ascorbic acid

DISCUSSION

Antioxidants act as a radical scavenger, a hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, an enzyme inhibitor, synergist, and metal-chelating agents. Both enzymatic and non-enzymatic antioxidants exist in the intracellular and extracellular environment to detoxify Reactive Oxygen Species (ROS) [12].

Anti-oxidants action takes place at four different levels such as preventive (which suppress the formation of free radicals e. g. Glutathione peroxidase, catalase); radical scavenging (that scavenges active radical to suppress chain reaction and break down the chain propagation, e. g. Vit. C, Vit. E), repair and de novo (that removes oxidative modified protein and prevent the accumulation of oxidized protein). Also, DNA repair system plays an important role, enzymes like glycosylase and nucleases that repair the damaged DNA; and adaptation (where the appropriate signal is given for production and transport of antioxidant to right site) [13].

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells [14]. The destructive action of hydroxyl radicals has been implicated in several neurological autoimmune diseases such as HIV associated with Neurocognitive disorder (HAND) when immune cells become over-activated and toxic to neighboring healthy cells [15]. Thus, the removing of H_2O_2 is very important for antioxidant defense in cell or food systems.

The aqueous extracts of *Solanum virginianum*, *Boerhavia diffusa*, and *Abutilon indicum* have shown significant H2O2 scavenging activity in dose dependent manner as compared to ascorbic acid.

DPPH is a well-known radical and acts as trap "scavenger" for other radicals. It is based on electron and H atom transfer. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. Because of a strong absorption band centered at about 520 nm, the DPPH radical has a deep violet color in solution and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm of the DPPH [16]. The aqueous extract of Group C (combination of all ten drugs) as well as Group A and *Tribulus terrestris* had shown antioxidant activity by DPPH scavenging assay in dose-dependent manner as compared to the standard drug.

The qualitative assay of antioxidant activity through HPTLC technique by using DPPH showed that the yellow zones indicating the compounds extracted with methanol have antioxidant activity. The degree of activity of all the samples tested can be determined qualitatively from observation of the yellow color intensity. Five compounds were separated in the S9 followed by three compounds in S6 and S8 samples respectively, while S2, S5 have not shown any active compound separation. The prominent compounds were found at 0.16, 0.17, 0.18, 0.21, 0.25, 0.27, and 0.61 Rf values.

Superoxide is biologically quite toxic and is deployed by the immune system to kill invading microorganisms. In phagocytes, superoxide is produced in large quantities by the enzyme NADPH oxidase for use in oxygen-dependent killing mechanisms of invading pathogens.

Superoxide scavenging activity was found to be good in the aqueous extract of *Abutilon indicum* while another sample of *Desmodium gangeticum* had shown least scavenging activity.

Ferric reducing antioxidant power is based on electron transfer reaction. This assay measures the reducing power of a potential antioxidant which reduces the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}) leading to the formation of a deep blue complex of Ferrous ion and 2,3,5,-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (Fe^{2+}/TPTZ). Higher FRAP value give higher antioxidant activity because FRAP value is based on reducing ferric ion where antioxidants act as reducing agent which can donate a single electron or hydrogen for reduction. Among the selected samples of Madhuraskandha, aqueous extracts of *Tribulus terrestris*, *Solanum xanthocarpum*, and *Asparagus racemosus*, *Boerhavia diffusa, Ricinus communis* have higher FRAP values in concentration dependent manner.

The aqueous extracts of the whole plant of *Solanum virginianum*, root of *Ricinus communis*, the stem of *Tinospora cordifolia*, tuberous root of *Asparagus racemosus* and the root of *Boerhavia diffusa* had been assessed for their antioxidant activity by FRAP and H_2O_2 scavenging activity. FRAP and DPPH scavenging activity had been evaluated for the aqueous extract of tuber of *Pueraria tuberosa*, the fruit of *Tribulus terrestris*, and leaves of *Leptadenia reticulata*. The root of *Desmodium gangeticum* had been studied for FRAP and Superoxide scavenging activity and the root of *Abutilon indicum* for Superoxide and H_2O_2 scavenging activity. All the three combinations (Gr A, Gr B and Gr C) were subjected to DPPH scavenging activity.

Abutilon indicum, Solanum xanthocarpum, Tribulus terrestris, Boerhavia diffusa, Group A and Group C had shown antioxidant activity by illustrated methods. *Pueraria tuberosa* had not shown antioxidant activity by DPPH or FRAP assay. *Asparagus racemosus, Ricinus communis and Tinospora cordifolia had shown moderate activity for FRAP and H2O2 scavenging activity. The least antioxidant activity was observed in Desmodium gangeticum by FRAP and Superoxide scavenging activity respectively. Leptadenia reticulata was reported for least antioxidant activity by FRAP assay and DPPH scavenging activity*.

The phytochemicals like anthocyanin, carbohydrates [17], flavonoids [18], carotenoids, phytosterol, phenolic acid, triterpenoids, saponin, xanthophyll, oregano sulfides, and stilbenes are responsible for antioxidant activity [19]. The preliminary phytochemical screening of methanolic and aqueous extracts showed the presence of carbohydrates, reducing sugar, amino acids, protein, tannin, steroids, flavonoids, anthraquinone, and saponin in the majority of drugs [5]. These constituents may be responsible for their antioxidant activity.

The above anti-oxidant agent decreases the destruction cell activity by radical scavenging behavior as well as electron donating capacity which promotes cell longevity which falls under the spectrum of *Rasayana* activity. Madhuraskandha includes the drugs either of *Madhurarasa* or *Rasa* oriented *vipaka* or *Prabhava*.

The drugs of *Madhura Rasa* are mainly attributed with *Balya* (improves strength), *Jeevaniya* (invigorating), *Preenana* (nourishing), *Brimhaniya* (increases muscle bulk of body), *Saptadhatuvardhana* (promotes the growth of all seven tissues) activities and which clearly indicates cell protective and promoting activities falling under anti-oxidant pharmacological profile.

CONCLUSION

Analysis of Madhuraskandha drugs through spectroscopic and chromatographic technique showed that they possess free radical scavenging activity. The drugs of Madhuraskandha can be a good source of natural antioxidants as Group C (combination often drugs) exhibit potent DPPH radical scavenging activity. The drugs like *Kantakari, Guduchi, Punarnava* having *Katu, Tikta* as dominant rasa, but they can perform their *Rasayana* Karma by *Madhuravipaka* or *Madhuraprabhava*.

CONFLICT OF INTERESTS

Declare none

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