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Research Article

COMPARATIVE BIOEFFICACY OF ANTIOXIDANT POTENTIAL OF FOURTEEN INDIGENOUS WOUND HEALING PLANTS

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ABSTRACT

Plants are organic chemist and provide excellent sources of therapeutic organic compounds. Application and administration of these plants and their products to external use in wound healing is an age old practice. Phytoingredients like flavonoids, polyphenols, alkaloids, terpenoids and tannins contribute antioxidant potential as they have inherent wound healing activity. Investigations have been carried out on *in vitro* antioxidant properties of fourteen indigenous wound healing plants, namely *Acalypha indica* Linn (leaves), *Azadirachta indica* A. Juss (leaves), *Aloe vera* Linn (stem), *Curcuma longa* Linn (rhizome), *Curcuma aromatica* Linn (rhizome), *Cynodon dactylon* Linn (leaves), *Coriandrum sativum* Linn (fruits), *Murraya koenigii* Spreng (root), *Pongamia glabra* Vent (leaves), *Sphaeranthus indicus* Linn (flower), *Terminalia arjuna* Roxb. (stem bark), *Tridax procumbens* Linn (leaves), *Vitex negundo* Linn (leaves), *Ziziphus jujuba* Mill (root). Assessment of the free radical scavenging potential of these plants has been worked out by standard methods in fresh aqueous juice of each plant, at a concentration of 0.1% (w/v). Enzymatic activities *viz.* catalase, peroxidase, super oxide dismutase, glutathione peroxidase and non enzymatic - reduced glutathione are taken as experimental parameters. Amongst them, *M. koenigii* exhibits excellent antioxidant activity, followed by *C. aromatica* and *A. vera*, whereas, *C. sativum* had relatively poor activity. Remaining eleven plants could demonstrate moderate potentiality. The validity of experimentation has been computed statistically by one way ANOVA and HASSE diagram considering *C .longa* as 100% effective drug and owing to its high ethnobotanical value. Formulation of fresh juice of above three plants would provide better efficacy to prevent oxidative stress.

Keywords: Antioxidant, *Murraya koenigii*, *Curcuma aromatica*, *Aloe vera*

INTRODUCTION

The role of free radicals and reactive oxygen species (ROS) has gained much attention in recent years because they are closely associated with many pathological conditions such as cancer, arthritis, cardiovascular diseases, liver diseases[1] and wound[2]. Some of these plant products have been used in different systems of medicines viz. Ayurveda, Unani and Homeopathy. Charak Samhita and Sushrut Samhita include 700 plants as sources of drugs. There are 1700 herbs have been mentioned in Materia Medica with their use and applications. A study of indigenous medicinal plants with special reference to wound healing properties has a wide scope for searching various chemical ingredients with high therapeutic index. Polyhydroxy flavones, flavonones, flavanols, isoflavones and many more phytoingredients have been proved to have a high grade of antioxidant activity. Proanthocyanidins are a special class of flavonoids, exhibiting potent antioxidant properties. Some of the important and well established antioxidants from plant source are Curcumin from Turmeric, Eugenol from clove, and Thymol from Thyme. Among the plant material berries[3] fruits[4], vegetables and spices are also reported for their richness in natural compounds as antioxidants. Comparative account of methanolic extract of leaves of four plants as potent antioxidative agents was reported[5]. Recently, the antioxidant activity of some plants of Cachar District of Assam (India) has been reported[6]. Many researchers published reviews on antioxidant activities of plant origin. Recently, we have reported 283 wound healing plants and out of them, fourteen possess high potential as wound healing agents in rat[7]. The names of shortlisted wound healing potential plants are: the leaves of *Acalypha indica* Linn , the leaves of *Aloe vera* Linn, the leaves of *Azadirachta indica* A. Juss, the fruits of *Coriandrum sativum* Linn, rhizomes of *Curcuma aromatica* Linn, rhizomes of *Curcuma longa* Linn, leaves of *Cynodon dactylon* Linn, roots of *Murraya koenigii* Spreng, the leaves of *Pongamia glabra* Vent, flowers of *Sphaeranthus indicus* Linn, stem bark of *Terminalia arjuna* Roxb, the leaves of *Tridax procumbens* Linn, leaves of *Vitex negundo* Linn and roots of *Ziziphus jujuba* Mill. As the antioxidants potential of the wound healing plants has not been critically evaluated so far, therefore the main objective of the present study is to investigate the free radical scavenging activities of these wound healing plants of North Maharashtra region using *in vitro* model.

MATERIALS AND METHODS

Collection of plant material

Plants were collected from North Maharashtra Region during the period of September, 2008 to January 2009. Specimens of the herbarium are stored in the Department of Zoology for future reference. The plants used for antioxidant activity in present work are listed in Table 1 and they belong to twelve families.

Table 1: A list of wound healing plants as the antioxidant

Name of plants	Family	Vernacular name	Herbarium No.	Parts used
Acalypha indica Linn	Euphorbiaceae	Khokali	Aox/ac-Z-1	Lf
Aloe vera Linn	Asphodelaceae	Korphad	Aox/av-Z-2	St
Azadirachta indica A.Juss	Meliaceae	Neem	Aox/az-Z-3	Lf
Coriandrum sativum Linn	Umbelliferae	Dhane	Aox/cs-Z-4	Fr
Curcuma aromatica Salisb	Zingiberaceae	Aambe halad	Aox/ca-Z-5	Rh
Curcuma longa Linn	Zingiberaceae	Halad	Aox/cl-Z-6	Rh
Cynodon dactylon Linn	Poieaceae	Durva	Aox/cd-Z-7	Lf
Murraya koenigii Spreng	Rutaceae	Kadhi patta	Aox/mk-Z-8	Rt
Pongamia glabra Vent	Fabaceae	Karanj	Aox/pg-Z-9	Lf
Sphaeranthus indicus Linn	Astreaceae	Gorakhmundi	Aox/si-Z-10	Fl
Terminalia arjuna Roxb	Combretaceae	Arjuna	Aox/ta-Z-11	Sb
Tridax procumbens Linn	Asteraceae	Ekdandi	Aox/tp-Z-12	Lf
Vitex negundo Linn	Verbenaceae	Nirgudi	Aox/vn-Z-13	Lf
Ziziphus jujuba Mill	Rhamnaceae	Bor	Aox/zj-Z-14	Rt

Lf= Leaf, Rh= Rhizome, Rt= Root, Fl= Flower, Sb= Stem bark, St= Stem

Preparation of extract

Each of the plant taken for the study was collected freshly from the local area and stored under refrigerated condition till use. The samples were prepared by grinding one gram each of the plant part in 2 ml of 0.1M phosphate buffer pH 7.0, separately, in a pre-chilled mortar and pestle. The extracts were centrifuged at 10,000 g at 4ºC for 10 minutes. The supernatants thus obtained were used within four hours for various enzymatic and non-enzymatic antioxidants assays.

Assay of superoxide dismutase (SOD) activity

The assay of superoxide dismutase was done according to the procedure of Das *et al*.[8]. In this method, 1.4 ml aliquots of the reaction mixture (comprising 1.11 ml of 50 mM phosphate buffer of pH 7.4, 0.075 ml of 20 mM L-Methionine, 0.04ml of 1% (v/v) Triton X-100, 0.075 ml of 10 mM Hydroxylamine hydrochloride and 0.1ml of 50 mM EDTA) was added to 100 µl of the sample extract and incubated at 30ºC for 5 minutes. 80 µl of 50 µM riboflavin was then added and the tubes were exposed for 10 min to 200 W-philips fluorescent lamps. After the exposure time, 1ml of Greiss reagent (mixture of equal volume of 1% sulphanilamide in 5% phosphoric acid) was added and the absorbance of the color formed was measured at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrite formation under assay conditions.

Assay of catalase activity

Catalase activity was assayed by the method of Sinha[9]. The enzyme extract (0.5 ml) was added to the reaction mixture containing 1ml of 0.01 M phosphate buffer (pH 7.0), 0.5 ml of 0.2 M H_2O_2, 0.4 ml H_2O and incubated for different time period. The reaction was terminated by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes and the absorbance was read at 610 nm. Catalase activity was expressed in terms of moles of H_2O_2 consumed/min/mg protein.

Assay of peroxidase activity

The assay was carried out by the method of Addy and Goodman[10]. The reaction mixture consisted of 3ml of buffered pyrogallol [0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0)] and 0.5 ml of 1% H_2O_2. To this added 0.1 ml enzyme extract and O.D. change was measured at 430 nm for every 30 seconds for 2 minutes. The peroxidase activity was calculated using an extinction coefficient of oxidized pyrogallol (4.5 litres/mol).

Assay of glutathione peroxidase (GPx) activity

Glutathione peroxidase was assayed according to the procedure of Rotruck *et al*.[11] with some modifications. The reaction mixture consisting of 0.4 ml of 0.4 M sodium phosphate buffer (pH 7.0), 0.1 ml of 10mM sodium azide, 0.2 ml of 4 mM reduced glutathione, 0.1 ml of 2.5 mM H_2O_2, 0.2 ml of water and 0.5 ml of enzyme was incubated at 0, 30, 60, 90 seconds respectively. The reaction was terminated with 0.5 ml of 10% TCA and after centrifugation; 2 ml of the supernatant was added to 3 ml of phosphate buffer and 1ml of DTNB reagent (0.04% DTNB in 1% sodium citrate). The color developed was read at 412 nm and the enzyme activity is expressed in terms of µg of glutathione utilized/min/mg protein.

Estimation of reduced glutathione (GSH)

The amount of reduced glutathione in the samples was estimated by the method of Boyne and Ellman[12]. 1ml of the sample extracts were treated with 4.0 ml of metaphosphoric acid precipitating solution (1.67 g of glacial metaphosphoric acid, 0.2 g EDTA and 30 g NaCl dissolved in 100ml water). After centrifugation, 2.0 ml of the protein-free supernatant was mixed with 0.2 ml of 0.4 M Na_2HPO_4 and 1.0 ml of DTNB reagent (40 mg DTNB in 100 ml of aqueous 1% tri sodium citrate). Absorbance was read at 412 nm within 2 minutes. GSH concentration was expressed as nmol/mg protein.

RESULTS

The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as SOD, CAT, GSH, etc. When the balance between ROS (reactive oxygen species) production and antioxidant defense is lost, 'oxidative stress' results, which through a series of events deregulates the cellular function leading to various pathological conditions. Enzymatic and nonenzymatic activities of the some medicinal plant are summarized in table 2. Catalase, peroxidase, SOD and GPx oxidoreducatase enzymes of plant origin are distributed in all plants and non enzymatic GSH activity. Here, we considered *C. longa* as a standard plant drug on the basis of earlier reports, as it contributes greatest number of publications related to the said activity. For the calculation purpose, this drug was considered to have 100% potentiality in order to validate the experimental parameters in question.

SOD

C. aromatica (320.55 %), *A. Vera* (280.16 %) and *M. koenigii* (244.72 %) possess significant ($p<0.01$) superoxide dismutase activity as compared with standard (fig 1). This is true for other plants also. *C. dactylon* (207.70 ± 3.48) and *T. procumbens* (153.70 ± 2.92) exhibit moderate activity. What is significant is that *Ac. indica* (115.10 ± 1.32), *S. indicus* (114.50 ± 1.36) and *Az. indica* (92.66 ± 1.15) showed parallel activity with standard plant (Table 2). Less activity was found in *Z. jujuba* (81.09 ± 0.085), *P. glabra* (72.52 ± 0.56), *C. sativum* (62.81 ± 0.50), *V. negundo* (50.75 ± 0.36) and *T. arjuna* (10.11± 0.09).

Catalase

The catalase activity is predominantly found significant ($p<0.01$) in *A. Vera* (323.90 ± 4.01), *C. aromatica* (237.00 ± 1.29), and *M. koenigii* (173.70 ± 6.05) as compared with *C. longa* (61.25 ± 2.54). Whereas, *T. arjuna* (113.40 ± 0.12), *Z. jujuba* (59.98 ± 0.27) possess moderate activity (Table 2). The *V. negundo* (39.56 ± 0.13), *P. glabra* (39.46 ± 0.27), *Ac. indica* (26.15 ± 0.42), *T. procumbens* (24.11 ± 0.61), *Az. indica* (17.36 ± 0.39), *S. indicus* (11.62 ± 1.61) and *C. dactylon* (1.38± 0.04) significantly decreased catalase activity ($p<0.01$). However, *C. sativum* (58.52 ± 1.87) had no significant activity.

Peroxidase

The peroxidase activity significantly ($p<0.01$) increased in the root of *M. koenigii* (26.46 ± 0.816) as compared with rhizome *C. longa* (8.21 ± 0.090). Moderately increased ($p<0.01$) activity was observed (Table 2) in *C. dactylon* (14.46 ± 0.199), *C. aromatica* (12.31 ± 0.337) and *Ac. indica* (10.25 ± 0.201), as compared with earlier plants. *S. indicus* (6.52 ± 0.033), *A. Vera* (6.43± 0.114), *Az. indica* (4.15 ± 0.029), *T. procumbens* (2.23 ± 0.026), *Z. jujuba* (2.01 ± 0.007), *V. negundo* (1.94 ± 0.008), *T. arjuna* (1.05 ± 0.007) and *C. sativum* (0.67 ± 0.002) showed significantly ($p<0.05$) decreased activity. However, the antioxidant value in *P. glabra* is more or less similar to that of *C. longa* (Table 2).

GPx

Glutathione peroxidase activity was effectively increased ($p<0.01$) in *A. Vera, C. aromatic* and *T. arjuna* that is 190.06 %, 185.03 % and 178.25 % respectively (fig 1) when compared with *C. longa* (100.00 %). However, *S. indicus, C. sativum, Az. indica* and *C. dactylo*n possess less activity. Whereas, *Z. jujuba* showed significantly ($p<0.05$) decreased activity. *Ac. indica, M. koenigii* and *V. negundo* exhibit non significant activity (Table 2)

GSH

It is evident from the table 2 and fig 1 that *Z. jujuba* (90.23 ± 1.08 %) significantly ($p<0.05$) decreased activity, whereas, rest of all plants possess excellent increased non enzymatic GSH activity (fig 1) as compared to *C. longa* (100.00 ± 0.00%).

HASSE diagram

The ranking of enzymatic and non enzymatic active plants was established by using a statistical tool, the HASSE diagram. The result is summarized in fig.2. It is clear from this *M. koenigii* holds first rank, followed by *A. vera* and *C. aromatica*.

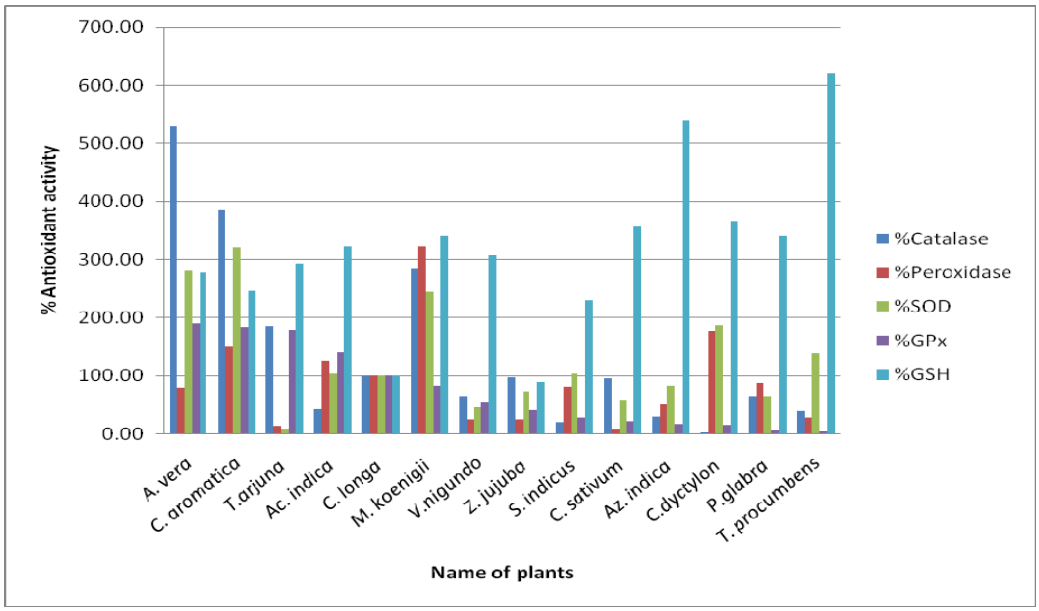

Fig. 1: Relative scores of as antioxidant activities of some wound healing plants

Table 2: Comparative account on enzymatic and non enzymatic antioxidant activities of fourteen wound healing medicinal plants

Name of plants	SOD (U/mg protein)	Catalase (U/mg protein)	Peroxidase (U/mg protein)	GPx (U/mg protein)	GSH (mg/g tissue)
C. longa	110.90 ± 1.55	61.25 ± 2.54	8.21 ± 0.090	42.94 ± 4.17	131.00 ± 1.13
Ac. indica	115.10 ± 1.32^{ns}	26.15 ± 0.42*	10.25 ± 0.201*	60.29 ± 5.46^{ns}	423.00 ± 1.16*
A. vera	310.7 0± 8.21*	323.90 ± 4.01*	6.43 ± 0.114*	81.61 ± 16.16*	364.10 ± 1.31*
Az. indica	92.66 ± 1.15^{ns}	17.36 ± 0.39*	4.15 ± 0.029*	7.27 ± 0.67*	707.00 ± 1.19*
C. sativum	62.81 ± 0.50*	58.52 ± 1.87^{ns}	0.67 ± 0.002*	8.82 ± 0.83*	467.20 ± 1.09*
C. aromatica	355.50 ± 13.55*	237.00 ± 1.29*	12.31 ± 0.337*	79.45 ± 2.25*	323.00 ± 1.14*
C. dactylon	207.70 ± 3.48*	1.38 ± 0.04*	14.46 ± 0.199*	6.51 ±0.76*	480.20 ± 1.02*
M. koenigii	271.40 ± 10.55*	173.70 ± 6.05*	26.46 ± 0.816	35.12 ± 3.93^{ns}	447.80 ± 8.38*
P. glabra	72.52 ± 0.56*	39.46 ± 0.27*	7.22 ± 0.017^{ns}	2.22 ± 0.15*	446.80 ± 2.76*
S. indicus	114 .50± 1.36^{ns}	11.62 ± 1.61*	6.52 ± 0.033*	11.67 ± 1.15*	301.40 ± 2.53*
T. arjuna	10.11 ±0.09*	113.40 ± 0.12*	1.05 ± 0.007*	76.54 ± 7.11*	384.30 ± 0.96*
T. procumbens	153.70 ± 2.92*	24.11 ± 0.61*	2.23 ± 0.026*	1.90 ± 0.36*	813.60 ± 1.18*
V.negundo	50.75 ±0.36*	39.56 ± 0.13*	1.94 ± 0.008*	23.11 ± 3.79^{ns}	401.70 ± 0.84*
Z. jujuba	81.09 ± 0.85*	59.98 ± 0.27^{ns}	2.01 ± 0.007*	17.72 ± 4.07**	118.20 ± 1.08**

Values expressed mean ± S.E., P values are compared with *C. longa*, * p<0.01, ** p<0.05, ns = non significant

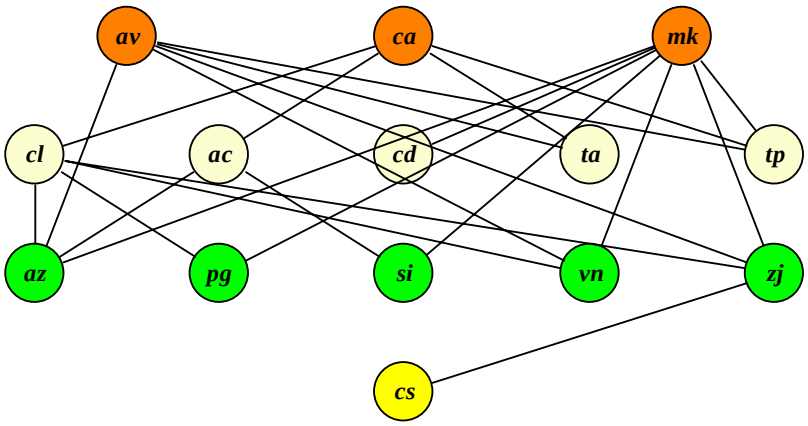

Fig. 2: HASSE diagram for prioritization of antioxidant plants

DISCUSSION

In India, common household recipes preparation includes leaves of *M. Koenigii*. The antioxidant property has been evaluated of this plant[13]. Mishra et al., demonstrated that the higher activity of reducing power and DPPH radical scavenging activity was found in methanolic extract of leaves of *M. koenigii*. They have further mentioned that it contains carbazole alkaloid that is koenigine and mahanimbine for the antioxidant activity. However, the difference is that they used leaves of *M. koenigii* and in present study we used roots of the *M. koenigii*. Therefore, we conclude that same principle(s) may be distributed throughout plant, hence root showed excellent antioxidant activity. Further more, phytochemical analysis also gave positive test to alkaloids and flavonoids. Our results are similar to the findings of Mishra et al.,[13]. Fifty percent hydroethanolic extract (hot and cold maceration) of *C. aromatica* showed potent antioxidant activity in DPPH. Nitric oxide radical scavenging reducing power and total antioxidant capacity, however aqueous extract exhibits poor antioxidant activity[14]. Our results are corroborate with the findings of Srividya et al.,[14] in contest to 50% hydroethanolic extract (hot and cold maceration), where as aqueous extracts lack the activity. This may be due to presence of flavones and flavonoids, in rhizome of *C. aromatica*. The antioxidant property may be lost during extraction in case of the experiment carried out by Srividya et al.,[14]. Our results on an aqueous juice of the same plant showed excellent activity, as it contains the flavonoids in the juice. Various studies have revealed that *A. vera* leaf skin possesses immense pharmacological activities and an antioxidant is must prominants[15]. They carried out experimental work on different fractions obtained from fresh leaves of *A. vera*. The hexane fraction possesses highest total antioxidant capacity and β-carotene bleaching antioxidant activity; followed by chloroform ethanol fractions high reducing power and DPPH radicals scavenging activity and these activities are attributed to phenolic compounds. Enzymes like Catalase, SOD, GPx and non enzyme GSH are present in the fresh aqueous juice of leaf of *A. vera*. These results are similar to those of Miladi and Damak[15]. Phenolic compounds are distributed not only in aqueous juice but also in all fractions. Miladi and Damak may be concluded that *A. vera* possesses total antioxidant activities. India has rich history for use of spices in the food. The antioxidant activity of Curcumin, a potent natural product was reported as early as 1975 to 2010[16]. It exhibits fourth rank in said antioxidant activities and results are verified. Balakrishnan et al.,[17] carried out Nitric Oxide Scavenging activity of the root of *Acalypha indica*. They demonstrated significant antioxidant activity *in vitro*. In the present study our results agreed with earlier researchers. Sultana et al.,[18] demonstrated considerable antioxidant action in methanolic extract of fruit of *C. sativum*, we agreed and justified the finding of them. We confirmed antioxidant activities of eight plants namely the fresh juice of leaves of *C. dactylon*[19],stem bark of *T. arjuna*[20], leaves of *T. procumbens*[21], leaves of *Az. Indica*[22], leaves of *P. glabra*[23], flowers of *S. indicus*[24], leaves of *V. negundo*[25] *and* root of *Z. jujuba*[26].They possess moderate antioxidant activity and our results are fortified the contents given by above authors. Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals[27]. Phenols from plants are a class of antioxidant agents which act as free radical terminators[28]. Thus, the present investigation reveals that the flavonoid is present in most of the plants, hence the order of potential antioxidant activity could be: *M. koenigii > A. vera > C. aromatica > Ac. indica > C. dactylon > T. arjuna > T. procumbens > Az. indica > P. glabra > S. indicus > V. negundo > Z. jujuba > C. sativum.*

CONCLUSION

In a nut shell, the most prominent plants like *M. koenigii, A. vera* and *C. aromatica* possess maximum antioxidant activity, since they contain phenolic and flavonoid group of natural principle(s). The formulation of these three plants in the form of fresh juice may be excellent supplement the nutritional food. We would like to propose assumption that the fresh aqueous juice from the root of *M. koenigii*, the rhizome of *C. aromatica* and the leaves of *A. vera* contain appreciable amounts of principle(s) and would enhance antioxidant activity synergistically. The presence of natural compounds like flavonoids, phenols, alkaloids, terpenoids and tannins may contribute to have free radical scavenging activity. We should not ignore use of these plants either in food or medicine.

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