COMPARATIVE BIOEFFECTIVENESS 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, Azadirachta indica A. Juss, Aloe vera Linn, Curcuma longa Linn, Curcuma aromatica Linn, Cynodon dactylon Linn, Diospyros lycioides, Endymion riparium, Ficus benghalensis, Gliricidia sepium, Haldea odorifera, Leptospermum robustum, Murraya koenigii Spreng, Pongamia glabra Linn, Sphaeranthus indicus Linn and Tithonia diversifolia. 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 has 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 and wound healing. Some of these plant products have been used in different systems of medicine viz. Ayurveda, Unani and Homeopathy. Charak Samhita and Sushrut Samhita include 700 plants as sources of drugs. The whole of these medicinal plants with species name to wound healing plants 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 well known and well established antioxidants from plant source are Curcumin from Turmeric, Eugenol from clove, and Thymol from Thyme. Among the plant material berries, fruits, 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. Recently, the antioxidant activity of some plants of Cachar District of Assam (India) has been reported. 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 rats. 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 Coriandrnum 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 Linn, 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

<table>
<thead>
<tr>
<th>Name of plants</th>
<th>Family</th>
<th>Vernacular name</th>
<th>Herbarium No.</th>
<th>Parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acalypha indica Linn</td>
<td>Euphorbiaceae</td>
<td>KhoKali</td>
<td>Aox/ac-Z-1</td>
<td>Lf</td>
</tr>
<tr>
<td>Aloe vera Linn</td>
<td>Asphodelaceae</td>
<td>Korphad</td>
<td>Aox/av-Z-2</td>
<td>St</td>
</tr>
<tr>
<td>Azadirachta indica A. Juss</td>
<td>Meliaceae</td>
<td>Neem</td>
<td>Aox/az-Z-3</td>
<td>Lf</td>
</tr>
<tr>
<td>Coriandrnum sativum Linn</td>
<td>Umbelliferae</td>
<td>Dhan</td>
<td>Aox/cs-Z-4</td>
<td>Fr</td>
</tr>
<tr>
<td>Curcuma aromatica Salisb</td>
<td>Zingiberaceae</td>
<td>Aambe halad</td>
<td>Aox/ca-Z-5</td>
<td>Rh</td>
</tr>
<tr>
<td>Curcuma longa Linn</td>
<td>Zingiberaceae</td>
<td>Halad</td>
<td>Aox/cl-Z-6</td>
<td>Rh</td>
</tr>
<tr>
<td>Cynodon dactylon Linn</td>
<td>Poiceae</td>
<td>Durva</td>
<td>Aox/ca-Z-7</td>
<td>Lf</td>
</tr>
<tr>
<td>Murraya koenigii Spreng</td>
<td>Rutaceae</td>
<td>Kadhi patta</td>
<td>Aox/mk-Z-8</td>
<td>Rt</td>
</tr>
<tr>
<td>Pongamia glabra Vent</td>
<td>Fabaceae</td>
<td>Karanj</td>
<td>Aox/pg-Z-9</td>
<td>Lf</td>
</tr>
<tr>
<td>Sphaeranthus indicus Linn</td>
<td>Astreaceae</td>
<td>Gorakmendi</td>
<td>Aox/si-Z-10</td>
<td>Fl</td>
</tr>
<tr>
<td>Terminalia arjuna Roxb</td>
<td>Combretaceae</td>
<td>Arjuna</td>
<td>Aox/tz-Z-11</td>
<td>Sb</td>
</tr>
<tr>
<td>Tridax procumbens Linn</td>
<td>Astreaceae</td>
<td>Ekdandi</td>
<td>Aox/ap-Z-12</td>
<td>Lf</td>
</tr>
<tr>
<td>Vitex negundo Linn</td>
<td>Verbenaceae</td>
<td>Nirgudi</td>
<td>Aox/vn-Z-13</td>
<td>Lf</td>
</tr>
<tr>
<td>Ziziphus jujuba Mill</td>
<td>Rhamnaceae</td>
<td>Bor</td>
<td>Aox/sj-Z-14</td>
<td>Rt</td>
</tr>
</tbody>
</table>

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. 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 0.1 ml 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 read at 510 nm. Catalase activity was expressed in terms of μg of catalase utilized/min/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 oxidoreductase 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, the drug was considered to have 100% potentiality in order to validate the experimental parameters in question.

SOD

C. aromatic (320.55%), A. Vera (280.16 %) and M. koenigii (24.72 %) 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 A. indica (115.10 ± 1.32), S. indicus (144.50 ± 1.36) and Az. indica (92.66 ± 1.15) showed parallel activity with standard plant (Table 2). Less activity was found in Z. jujuba (810.9 ± 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 predominantly found significant (p<0.01) in A. Vera (323.90 ± 4.01), C. aromatic (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 (39.5 ± 0.13), P. glabra (39.46 ± 0.27), A. 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 (3.58 ± 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. aromatic (12.31 ± 0.337) and A. 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 ± 0.02) 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 assayed according to the procedure of Rotruck et al. 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 H2O2, 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.

Assay of glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was assayed according to the procedure of Rotruck et al. 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 H2O2, 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 Elman. 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 ml of the protein-free supernatant was mixed with 0.2 ml of 0.4 M Na2HPO4 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.

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 ± 0.0%).

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. aromatic.

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

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

<table>
<thead>
<tr>
<th>Name of plants</th>
<th>SOD (U/mg protein)</th>
<th>Catalase (U/mg protein)</th>
<th>Peroxidase (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
<th>GSH (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. longa</td>
<td>110.90 ± 1.55</td>
<td>61.25 ± 2.54</td>
<td>8.21 ± 0.090</td>
<td>429.4 ± 4.17</td>
<td>131.00 ± 1.13</td>
</tr>
<tr>
<td>Ac. indica</td>
<td>115.10 ± 1.32*</td>
<td>26.15 ± 0.42*</td>
<td>10.25 ± 0.201*</td>
<td>60.29 ± 5.46*</td>
<td>423.00 ± 1.16*</td>
</tr>
<tr>
<td>A. vera</td>
<td>310.7 ± 8.21*</td>
<td>323.90 ± 4.01*</td>
<td>6.43 ± 0.114*</td>
<td>81.61 ± 16.16*</td>
<td>364.10 ± 1.31*</td>
</tr>
<tr>
<td>Az. indica</td>
<td>92.66 ± 1.15*</td>
<td>17.36 ± 0.39*</td>
<td>4.15 ± 0.029*</td>
<td>7.27 ± 0.67*</td>
<td>707.00 ± 1.19*</td>
</tr>
<tr>
<td>C. sativum</td>
<td>62.81 ± 0.50*</td>
<td>58.52 ± 1.87*</td>
<td>0.67 ± 0.002*</td>
<td>8.82 ± 0.83*</td>
<td>467.20 ± 1.09*</td>
</tr>
<tr>
<td>C. aromatica</td>
<td>355.50 ± 13.55*</td>
<td>237.00 ± 1.29*</td>
<td>12.31 ± 0.337*</td>
<td>79.45 ± 2.25*</td>
<td>323.00 ± 1.14*</td>
</tr>
<tr>
<td>C. dactylon</td>
<td>207.70 ± 3.48*</td>
<td>1.38 ± 0.04*</td>
<td>14.46 ± 0.199*</td>
<td>6.51 ± 0.76*</td>
<td>480.20 ± 1.02*</td>
</tr>
<tr>
<td>M. koenigii</td>
<td>271.40 ± 10.55*</td>
<td>173.70 ± 6.05*</td>
<td>26.46 ± 0.016</td>
<td>35.13 ± 3.93*</td>
<td>447.80 ± 0.33*</td>
</tr>
<tr>
<td>P. glabra</td>
<td>72.52 ± 0.56*</td>
<td>39.46 ± 0.27*</td>
<td>7.22 ± 0.017*</td>
<td>2.22 ± 0.15*</td>
<td>446.80 ± 2.76*</td>
</tr>
<tr>
<td>S. indicus</td>
<td>114.50 ± 1.36*</td>
<td>11.62 ± 1.61*</td>
<td>6.52 ± 0.033*</td>
<td>11.67 ± 1.15*</td>
<td>301.40 ± 2.53*</td>
</tr>
<tr>
<td>T. arjuna</td>
<td>10.11 ± 0.09*</td>
<td>113.40 ± 0.12*</td>
<td>1.05 ± 0.007*</td>
<td>76.54 ± 7.11*</td>
<td>384.30 ± 0.96*</td>
</tr>
<tr>
<td>T. procumbens</td>
<td>153.70 ± 2.92*</td>
<td>24.11 ± 0.61*</td>
<td>2.23 ± 0.026*</td>
<td>1.90 ± 0.36*</td>
<td>813.60 ± 1.18*</td>
</tr>
<tr>
<td>V. negundo</td>
<td>50.75 ± 0.36*</td>
<td>39.56 ± 0.13*</td>
<td>1.94 ± 0.008*</td>
<td>23.11 ± 3.79*</td>
<td>401.70 ± 0.84*</td>
</tr>
<tr>
<td>Z. jujuba</td>
<td>81.09 ± 0.85*</td>
<td>59.90 ± 0.27*</td>
<td>2.01 ± 0.007*</td>
<td>17.72 ± 4.07**</td>
<td>118.20 ± 1.00**</td>
</tr>
</tbody>
</table>

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

In India, common household recipes preparation includes leaves of M. koenigii. The antioxidant potential of this plant has been evaluated in methanolic extract of leaves of M. koenigii. They have further mentioned that it contains carbazole alkaloid that is koenigne 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.,15 Fifty percent hydroethanolic extract (hot and cold maceration) of C. aromatica showed potent antioxidant activity in DPH. Nitric oxide radical scavenging reducing power and total antioxidant capacity; however aqueous extract exhibits poor antioxidant activity.15 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 vera leaf skin possesses immense pharmacological activities and an antioxidant is must prominent.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 ?-carotene bleaching antioxidant activity; followed by chloroform ethanol fractions high reducing power and DPH 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.17 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 activity in methanolic extract of fruit of S. sativum, we agreed and justified the finding of them. We confirmed antioxidant activities of eight plants namely the fresh juice of leaves of C. dactylon19, stem bark of T. arjuna, leaves of T. procumbens20, leaves of Az. Indica21, leaves of P. glabra22, Flowers of S. indicus22, leaves of V. negundo23 and root of Z. jujuba24. 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 radicals27 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 formation 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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