**In vitro antioxidant, anti-inflammatory, thrombolytic potential of Drimia nagarjuna**, a Tribal medicinal plant from South India

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**Abstract**  
The present investigation was aimed to evaluate in vitro bioactivities (antioxidant, anti-inflammatory and thrombolytic) of Drimia nagarjuna. Leaf and bulb of the plant were extracted sequentially with nonpolar to polar solvents (Hexane> Chloroform> Ethyl acetate> Methanol> Water). Preliminary screening of phytochemicals revealed the presence of phenols, saponins, terpenoids, cardiac glycosides, sterols, phlobatannins, alkaloids and flavonoids. The reducing power of chloroform bulb extract increases with increasing concentrations and it showed highest total phenolic content (TPC) (9.87 ± 0.34 mg GAE /g extract) and total flavonoid content (TFC) (3.33±0.42 mg QE /g extract). The IC50 values of chloroform bulb extract for DPPH and ABTS scavenging activities were 8.79±0.89 µg/ml and 9.22±0.72 µg/ml respectively. The chloroform bulb extract also showed good correlation between TPC /DPPH (r=0.95) and TPC/ ABTS (r=0.96). The above extract exhibited potent anti-inflammatory activity with a maximum inhibition of 82.97% ±1.16 at 100 µg/mL which was found to be significant (p<0.006) when compared to diclofenac standard. Methanolic extract of both leaf and bulb showed thrombolytic activity with a maximum clot lysis 45.92% ±1.43 in bulb extract at 100 µg/mL and was significant (p<0.0001) with streptokinase (a positive control). UV/Visible and ATR-IR spectrum analysis revealed the presence of phenolic groups which might be principal active compounds for the bioactivities. The present study promisingly revealed that D.nagarjuna possesses potent antioxidant, anti-inflammatory and thrombolytic activities, which should be further explored for the identification of bioactive compounds.

**Keywords**: Antioxidant, Anti-inflammatory, Thrombolytic activity, Drimia nagarjuna and ATR-IR

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**Introduction**  
The physiological and biochemical processes in the human body produce free radicals and other reactive oxygen species (ROS) as byproducts. The imbalance between the generation of ROS and inactivation of ROS causes oxidative stress in organisms. Superoxide anion (O$_2^-$) is an important source of oxidative stress in abnormal platelet function and dysfunctional endothelium dependent vasodilation (Freedman, 2008). The excessive release of superoxide in blood vessels suppresses the activity of nitric oxide (NO) in platelets. The impaired platelet-derived NO release influences platelet recruitment to the growing thrombus which stimulates the inflammatory responses which is likely to be associated with acute coronary and stroke syndromes (Passkow, 2011). Therefore, the drugs which can scavenge ROS, inhibits the inflammation and thrombolytic activities, might be the best choice for cardiovascular diseases.

Tribal medicine is the mother of all the systems of medicine existing on Earth. Unlike Ayurveda, Siddha and Unani, the tribal medicine is not a codified
system, but the knowledge of this system was carried out generation to generation (Koppula, 2011). The genus Drimia (syn. Urginea) includes about 100 species, distributed around equatorial regions and in India (Stedje, 1987). Ancient Egyptians used these plants for the treatment of edema, emesis and cough. Romans also used Drimia bulb extracts as a cardiac tonic (Kameswari et al., 2012). Drimia nagarjunae Hemadri et Swahari, is a tribal medicinal plant from South India and is used ethnopharmacologically to treat breast abscess and cure piles (Koppula, 2011). The aim of the present investigation was to evaluate the antioxidant, anti-inflammatory and thrombolytic potential of D. nagarjunae.

Materials and Methods

Collection and extraction of plant material

The plant was collected from Bhata village, Sri Potti Ramulu Nellore district, Andhra Pradesh, India and was authenticated by National Ayurveda Dietetics Research Institute, Bangalore with authentication number SMPU/NADRI/ BNG/2013-14/83. The plant material (bulb and leaf) was dried and powdered. The dried samples were kept in airtight containers until further use. The powdered samples were extracted sequentially (Hexane > Chloroform > Ethyl acetate > Methanol > Water) at 1:10 (w/v) concentrations using a Soxhlet apparatus. The extract was filtered through Whatman No.1 filter paper and the filtrate collected. The filtrate was concentrated by rotary evaporator, stored at 4°C and used for further studies.

Phytochemical analysis

The plant extracts were screened for the presence of tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, alkaloids and phenols (Harborne, 1973).

Estimation of total phenolic total flavonoid content

Total phenolic content (TPC) was determined according to standard procedure with minor modifications (Singelton et al., 1999). The absorbance was measured at 765nm using double beam UV-VIS spectrophotometer (UV-1800 Shimadzu). TPC was expressed as mg gallic acid equivalents per gram of sample (mg GAE g^-1). Total flavonoid content (TFC) was determined by aluminium chloride method with slight modifications (Arya et al., 2012). The absorbance was recorded at 415 nm and TFC was expressed as mg quercetin equivalents per gram of the sample (mg QE g^-1).

Antioxidant activity

DPPH radical scavenging activity

The radical scavenging activity of the plant extracts was studied using 2, 2-diphenyl-1-picyrylhydrazyl (DPPH) radical scavenging method with slight modifications. One mL of plant extracts of different concentrations (2-20 μg/mL) was added to two mL of methanolic solution of DPPH (0.0025%). The assay mixture was incubated in dark at 37ºC for 30 mins. The decrease in absorbance of each solution was measured at 518 nm using UV-VIS spectrophotometer (UV-1800, Shimadzu). Ascorbic acid, a stable antioxidant was used as positive control (Blois, 1958). The percentage (%) of radical scavenging activity was calculated by the following formula:

\[
\text{Scavenging activity} \% = \frac{\text{AC} - \text{AS}}{\text{AC}} \times 100,
\]

where \(\text{AC}\) = Absorbance of sample, \(\text{AS}\) = Absorbance of control

ABTS\(^{\bullet+}\) assay

The antioxidant capacity of the plant extracts was estimated in terms of the ABTS\(^{\bullet+}\) scavenging activity with slight modifications. Briefly, ABTS\(^{\bullet+}\) was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium per sulphate and the mixture was allowed to react for 12 h at room temperature in the dark. The resulting solution was then diluted with 5 mM
phosphate-buffered saline (pH 7.4). One mL of plant extracts of different concentrations (2-20 μg/mL) was added to 0.5 mL of the diluted ABTS●+ solution, incubated for 20 min and the absorbance was measured at 745 nm (Re et al., 1999). The percentage (%) of radical scavenging activity was calculated by the following formula:

Scavenging activity (%) = AC – AS/AC X 100, where, AS= Absorbance of sample, AC= Absorbance of control

Anti-inflammatory activity

Anti-inflammatory activity of D. nagarjuna extracts was evaluated by protein denaturation method (Elias and Rao, 1988). Percentage inhibition of denaturation was calculated by using the following formula:

Inhibition (%) = (At-Ac) / Ac * 100, where, Ac= Absorbance of control, At= absorbance of test sample

Thrombolytic activity

Experiments for thrombolytic activity were carried by clot lysis method (Prasad et al., 2006). In brief, four mL of venous blood was drawn from healthy human volunteers (n = 50) without history of oral contraceptive or anticoagulant therapy (using a protocol approved by the institutional ethics committee of Kempegowda Institute of Medical Sciences, Bangalore with registration No. ECR/216/Inst/Kar/2013). An earlier consent was taken from healthy human volunteers, KIMS for collection of blood samples.500μl of blood was transferred to each of the previously weighed sterile micro centrifuge and incubated at 37°C for 45 min. To the commercially available lyophilised streptokinase vial (1500000 IU), five mL sterile distilled water was added and mixed properly. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of the tube alone). To each micro centrifuge tube containing pre-weighed clot, 100 1 of D. nagarjuna methanolic extracts were added. As a positive control, 100 1 of streptokinase and as a negative control 100 1 of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After the incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was performed with the blood samples of the 50 volunteers.

UV- Visible Spectra analysis

The chloroform bulb extract was centrifuged at 5000 rpm for 10 min and filtered. The filtrate was diluted to 1:10 ratio with methanol. The prepared extract having bioactive compounds were analysed by using UV-VIS spectrophotometer (UV-1800 Shimadzu), wavelength ranging from 190 nm to 1100 nm (Krishnaveni et al., 2012).

Attenuated total reflectance infra-red (ATR-IR) analysis - Chloroform bulb extract was subjected to ATR-IR (Bruker Alpha ECO-ATR spectrometer) analysis for the functional groups. A small amount of sample was placed directly on the germanium piece of the IR spectrometer with constant pressure and wave number ranged from 4000 cm⁻¹ to 675 cm⁻¹. The characteristic peaks were determined according to their functional groups (Beatriz, 2006).

Statistical analysis

All data were expressed as mean ±S.E of three replicates (n=3). Test significant differences were determined by two way ANOVA using Graph Pad Prism 6 and the values were considered to be significant (P<0.05).
Table – 1. Phytochemical screening of bulb and leaf extracts of *D. nagarjuna* 

<table>
<thead>
<tr>
<th>Test group</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>leaf</td>
<td>bulb</td>
<td>leaf</td>
<td>bulb</td>
<td>leaf</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sterols</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = present; - = absent

Table – 2. Total phenolic and flavonoids contents of bulb and leaf extracts of *D. nagarjuna*

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenolic (mg GAE/g)</th>
<th>Total flavonoids (mg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>leaf</td>
<td>bulb</td>
</tr>
<tr>
<td>Hexane</td>
<td>4.33±0.27</td>
<td>5.27±0.41</td>
</tr>
<tr>
<td>Chloroform</td>
<td>8.74±0.16</td>
<td>9.87±0.34</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>6.42±0.17</td>
<td>6.53±0.27</td>
</tr>
<tr>
<td>Methanol</td>
<td>3.97±0.38</td>
<td>4.18±0.33</td>
</tr>
<tr>
<td>Water</td>
<td>2.51±0.51</td>
<td>3.07±0.24</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SE. mg GAE/g: mg gallic acid equivalent per g of extract; mg QE/g: mg of quercetin equivalent per g of extract.

Results and Discussion

Phytochemical screening of *D. nagarjuna* leaves and bulb extracts revealed the presence of tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, alkaloids and phenolic compounds (Table - 1). Table 2 revealed that the chloroform bulb extract showed the highest amount of TPC (8.74±0.16 mg/GAE/g of extract) and TFC (3.33±0.42 mg/QE/g extract) whereas chloroform leaf extracts exhibited TPC (8.74±0.16 mg/GAE/g of extract) and TFC (2.67 ±0.38 mg/QE/g extract). The leaf and bulb extracts of the plant showed a concentration dependent scavenging activity on both DPPH and ABTS** radicals. Among the extracts, chloroform bulb extract exhibited best scavenging activity on DPPH and ABTS** radicals. The lower IC₅₀ values for DPPH (8.79±0.89 µg/mL) (Fig.-1) and ABTS** (9.22±0.72 µg/mL) (Fig.-2)
corresponds to higher scavenging activity and this result is in accordance to the earlier studies (Maisuthisakul et al., 2007). Present findings showed good correlation between TPC/DPPH (r=0.95) and TPC/ABTS+ (r=0.96) of chloroform extract of bulb. Among all the extracts, chloroform bulb exhibited maximum albumin denaturation inhibition of 82.97 ± 1.16 % at 100µg/mL and was found to be significant (p<0.006) with diclofenac as standard (Fig.3). A positive correlation (r = 0.98) was observed between TPC of chloroform extracts of bulb and its anti-inflammatory activity confirming that the phenolic compounds might be the factor to be responsible for anti-inflammatory activity. The methanolic leaf and bulb extracts of the plant exhibited 37.52% ± 2.28 and 45.92%±1.43 clot lysis after incubation at 37°C for 90 min. Whereas Streptokinase showed 56.198%±1.03 and distilled water 8.3% ± 0.5 clot lysis (Fig.4). The mean difference in percentage (%) clot lysis between Streptokinase and bulb extract was found to be significant (p <0.001).

UV-visible spectrum of chloroform bulb, methanolic bulb and methanolic leaf extracts showed the absorbance between 190 nm to 350 nm (Fig. 5). The spectra peaks in the range of 210 – 310 nm are due to the phenolic groups and those in the range of 255 – 280 nm are specific for flavonoids (Manole, 2008). ATR-IR analysis of chloroform bulb, methanolic bulb and methanolic leaf extracts confirmed the presence of phenol, carboxylic acid and aromatics (Beatriz, 2006) (Fig 6). The UV/Visible spectrum and ATR-IR analysis revealed presence of phenolic groups which might be...
principal active compounds. The polyphenols, quercetin and catechin synergistically act in reducing platelet recruitment via inhibition of PKC-dependent NAD (P) H oxidase activation (Cook et al., 1996). Many phenolic compounds, particularly flavonoids exhibit a wide range of scavenging effect. The anti-oxidants indirectly inhibit platelet aggregation through scavenging of reactive oxygen species, thus producing NO, thereby inhibiting the platelet aggregation (Pignatelli et al., 2006). Due to inhibition of platelet aggregation, the inflammation and athero- thrombosis may be prevented. Similarly, D. nagarjunae extracts showed potent antioxidant, anti-inflammatory and thrombolytic activities, which should be further explored for the development of drug for oxidative stress related diseases.

Conclusion

In conclusion, the present study promisingly revealed that D. nagarjunae can be an effective potential source of novel antioxidant, anti-inflammatory and thrombolytic drugs. There may be a possibility of obtaining a single bioactive compound after bioassay guided purification which might cure oxidative stress related diseases.

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References


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