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In-vitro anti-inflammatory and anti-oxidant study of *Sansevieria cylindrica* Bojer ex. Hook and *Plumeria obtusa* L. plants using different methods

Research Article

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Abstract

Background: *Sansevieria cylindrica* and *Plumeria obtusa* are used as a medicinal plant during intentional and unintentional accidental injuries. However, limited investigations have been performed to study pharmacological activities of these plants. Objectives: The current study designed at examining *in-vitro* anti-inflammatory and anti-oxidant effect of both plant extracts. Material & Method: Initially, the collection and authentication of both the plants performed. Phytochemical screening was done thereafter. *Sansevieria cylindrica* leaves and *Plumeria obtusa* seed pods were extracted using a combination of water and ethanol. Anti-inflammatory effect was assessed using membrane stabilization and protein denaturation assays. Anti-oxidant activity was measured by free radicals scavenging method using different reactive oxygen species producing reagents. Results: The dose dependent increase in anti-inflammatory and anti-oxidant activities were reported by both plants. Overall, *Sansevieria cylindrica* has shown higher rate of prevention of inflammation and oxidation compared to *Plumeria obtusa* extract. Both plants showed comparable anti-inflammatory and anti-oxidant activity in combination with that of reference drugs. Conclusion: The hydro-alcoholic extracts of *Sansevieria cylindrica* and *Plumeria obtusa* individually and also as 1:1 blend might be responsible for an anti-inflammatory and anti-oxidant activities.

Key Words: *Sansevieria cylindrica, Plumeria obtusa,* Albumin denaturation, Anti-inflammatory, Anti-oxidant.

Introduction

An Inflammation is ordinary protective response to tissue damage brought on by physical trauma and hazardous substances. In addition, inflammation is linked to pain and entails, among other things raised in protein denaturation, an elevation in membrane modification and vascular permeability (1,2). Non-steroidal ant-inflammatory drugs (NSAID) are often used in the treatment of inflammatory illnesses. These drugs have a number of side effects, especially gastrointestinal irritation, which can lead to stomach ulcer (3). As a result, the search for natural sources, specifically phytochemicals with anti-inflammatory potential, has significantly increased in recent years (4).

A chemical that can neutralize the majority of reactive oxygen species (ROS), including peroxyl (ROO) radicals, reactive hydroxyl (OH) radicals, superoxide (O_2), hydrogen peroxide (H_2O_2), peroxynitrite and nitric oxide (NO) anions are known as an anti-oxidant (ONOO) (5). An excessive amount of free radicals can results in to oxidative damage to biomolecules (proteins, lipids and DNA components), which can then cause many chronic diseases, including atherosclerosis, rheumatoid arthritis, chronic inflammation, cardiovascular diseases, stroke, and septic shock (6). Synthetic antioxidants that are now in the market, such as tertiary butylated hydroquinones (TBHQ), Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been linked to adverse health effects (7). Hence, the demand for use of synthetic anti-oxidants has reduced nowadays whereas a trend to substitute them with natural products has increased (8). One of the newest areas of medicine is complementary and alternative medicine. Indians and other tribes have been familiar with the use of plants as medicine since 5000 B.C. (9). Across the globe, many plants are used for their therapeutic capabilities against a variety of ailments in either basic plant extracts or its formulations (10). Use of plant base medicines as one of potential anti-oxidant sources for prevention of oxidation of important bioactive molecules have been reported (11).

The *Sansevieria cylindrica* plant, is a member of the Asparagaceae family, and is most frequently found in Africa. It is also used for ornamental purposes in India, Egypt and other nations (12, 13). Numerous

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biological actions reported by Sansevieria species includes antibacterial, free radical scavenging, antidiabetic, anticancer, and capillary permeability suppression (14). The Apocynaceae family includes the *Plumeria obtusa* plant, which is found in Florida, Greater Antilles, Northern, Central America and southern Mexico (15). The Plumeria is used as an emmenagogue, rubefacient, stimulant, febrifuge, hemostatic, purgative, laxative, and vermifuge among many more medical purposes (16,17).

There are very few reports that demonstrate their biological activities. In our research, it has been observed that, hydro-alcoholic extracts of these plants contained good amount of phenolic and flavonoids compounds that might explain their pharmacological activities. This projected research was aimed to assess *in-vitro* anti-inflammatory and anti-oxidant capacity by free radical scavenging method using hydro-alcoholic extracts of the plants.

Materials and Methods

Collection and authentication of plant material

The fresh whole plants of *Sansevieria cylindrica* and *Plumeria obtusa* were collected from Akole (Mehenduri), Ahmednagar, Maharashtra, India. The leaves and seed pod of plants were washed to remove dust and other impurities. The herbariums of plants were submitted to Botanical Survey of India (BSI), Pune. For long-term storage, the samples were air-tight packaged after being shade dried and ground into powder using an electrical grinder.

Hydro-alcohol soluble extraction

The leaves and seed pod sample in the powder form were weighed accurately and solubalized in 100 ml solvent [Water (H_2O) + Ethanol (C_2H_6O); 40:60 units]. The drugs were loaded on rotary-shaker for 24 hrs. at 100 rpm at room temperature. Then filtrate (25 ml) was transfer in accurately weighed porcelain dish. After that, it was allowed to evaporate for drying. The changes in weight were determined and extractive values calculations were expressed in percent w/w (18).

Qualitative and quantitative phytochemical analysis

The qualitative phytochemical screening of extracted plant material was executed by standard method (18). Various tests were conducted to determine existence or lack of primary metabolites viz; proteins, carbohydrates, fats, fixed oils and secondary metabolites viz alkaloids, glycosides, flavonoids, saponins and tannins. Metabolites were also quantitatively determined.

Assessment of total phenolic content

The current study verifies total phenolic content in samples by using the Folin-Ciocalteu reagent (19). Folin-Ciocalteu reagent (2.5 ml, 1:10 dilution) and sodium carbonate (2 ml, 7.5% w/v) were added to plant extracts (0.5 ml) using concentrations ranging from 20 to 120 µg/ml. It was then incubated for 15 minutes (min.) at normal temperature. Further the absorbance was documented using a UV-visible spectrophotometer at 750 nm. Gallic acid (reference standard) was dissolved in methanol and results were stated as microgram (µg) of Gallic acid equivalent/ milligram (mg) of extract sample.

Assessment of total flavonoid content

The method reported earlier by Maswada *et.al.* was used to determine total flavonoid content of extracts by colorimetry (20). Extracts and Quercetin used as a standard (10 to 60 µg/ml) were added to distilled water (4 ml) in a 10 ml volumetric flask. To this flask, after each 5 min break, 5% sodium nitrate (0.3 ml), 10% Aluminium chloride (0.3 ml) and 1M sodium hydroxide (2 ml) were added and then a volume made up to 10 ml with solvent. The absorbance of this mixture was measured at 510 nm against blank. All reported results were documented as µg of Quercetin equivalent/ mg of extract.

Assessment of *in-vitro* anti-inflammatory activity

Protein denaturation inhibition study

Preparation of reference drug (positive control)

To assess an anti-inflammatory effects of plant extracts, protocol described by Dharmadeva *et.al.* (21) and Nguemnang *et.al.* (22) was used with very minor modifications. Ibuprofen and Diclofenac were used as a reference standard. Diclofenac tablet was compressed to get fine powder. The Diclofenac powder (0.2 g) was added to distilled water (20 ml). This was mix well by a vortex to get a clear solution. A similar process was followed for preparation of Ibuprofen as a reference control.

Study procedure

The 1 ml of Diclofenac sodium and Ibuprofen at concentrations of 0.2 to 1.0 mg/ml and herbal extracts were homogenised with bovine serum albumin (BSA) (5%, 1 ml) and then incubated for 15 min at 27°C. The mixture of BSA and distilled water was used as negative control. The mixture was kept in water bath at 70°C for 10 min. This will allow denaturation of the protein. The absorbance of each reaction mixture was measured at 680 nm after it is being cooled to room temperature. The percentage inhibition of protein denaturation was determined by;

% inhibition = (OD of control − OD of extract/OD of control) × 100

Membrane stabilization

Hypotonicity-induced hemolysis

Hypotonicity-induced hemolysis was studied with some modifications as per the standard procedure (11). The blood sample was obtained from a healthy male rat prior to experimentation. It was centrifuged (3000 rpm) until 10 min. and then washed three times with equal amount of saline solution. The plant extracts and reference sample (0.2-1.0 mg/ml) were individually mixed up with phosphate buffer (1 ml, pH: 7.0), hyposaline (2 ml) and red blood cells (RBCs) suspension (0.5 ml). For control, only saline solution

was used. All reaction mixtures were incubated (37°C) for 30 min. and then centrifuge (3000 rpm). The uppermost layer was transfer and absorbance was measured at 560 nm.

Heat-induced hemolysis

Heat-induced hemolysis was performed as per standard procedure by Rastogi *et.al.* with little modification (11). The reaction mixture (2 ml) comprised of plant extracts (1 ml) at different concentration range (0.2-1.0 mg/ml) and RBCs suspension (1 ml, 10%). For control, only saline solution was used. Aspirin was used as standard control. The reaction mixture was incubated (30 min., 56°C) in water bath. The reaction was stopped by cooling it below running tap water followed by centrifugation (2500 rpm, 5 min.). The supernatant was used for determining absorbance at 560 nm. Each of the anti-inflammatory procedure was perform in triplicates to get accurate results. Similar procedure was followed for 1:1 combination of plant extracts. Measurements of hemolysis was determined as per above given formula.

***In-vitro* anti-oxidant activity**

Assessment of *in-vitro* anti-oxidant potential of plants extracts was carried out using different assay methods illustrated by Hebbani *et.al.* (23) and Bhatti *et.al.* (24) with minor amendments. The assay procedures are described below;

H_2O_2 scavenging activity

To 3.4 ml of plant extracts (0.2-1.0 mg/ml), hydrogen peroxide (0.6 ml) was transfer and incubated for 10 min. at normal temperature. BHA was used as a standard for comparison. The absorbance of H_2O_2 upon oxidation was examine at 230 nm against blank (phosphate buffer alone) spectrophotometrically. Then percentage inhibition was calculated as below;

% inhibition = (OD of control − OD of extract/OD of control) × 100

Nitrous oxide (NO) radical scavenging activity

It was assessed by colorimetric method. In this method 200 µL of sodium nitroprusside (SNP) was mixed with 800 µL of the various concentration of extracts (0.2-1.0 mg/ml), which were dissolved in phosphate-buffered saline (25 mM, pH 7.4) and each mixture was incubated for 2.5 hrs. at 37°C under visible light exposure and further each sample was kept in dark for 20 min. at normal temperature. Subsequently, 300 µl Griess reagent (1% sulfanilamide + 0.1% naphthyl ethylene diamine hydrochloride in 2% H_3PO_4) was transferred to all samples and incubated (40 min). Color intensity of samples was measured at 546 nm against blank (2 ml H_2O and 0.6 ml Griess reagent). Ascorbic acid (reference standard) was used for comparative study. 200 µL SNP, 800 µL H_2O and 300 µL Griess reagent was used as negative control. The percentage inhibition was measured using above given formula.

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

Anti-oxidant activity of test samples was evaluated with minor modifications on the basis of DPPH free radical scavenging activity. To 2 ml of each concentration of extracts taken (0.2-1.0 mg/ml) 1 ml of DPPH solution (125 µM solubilized in methanol) was added. The mixture was well shaken and placed in dark area at 37°C for 30 min. and absorbance was reported at 517 nm against blank (Methanol: 2ml and DPPH: 1ml). Ascorbic acid solutions were prepared at same concentration ranges as a reference standard. Percentage inhibition was measured by comparing tests with control using above given formula.

Reductive ability

The various concentrations of 1 ml herbal extracts ranging from 0.2 to 1.0 mg/ml and phosphate buffer (pH 6.6; 0.2 M) were mixed with 1% potassium ferricyanide (1.5 ml) appropriately and then mixture was incubated for 20 min. at 50°C. 10% trichloroacetic acid (1 ml) was added to stop the reaction followed by centrifugation at 3000 rpm for 10 min. To the supernatant (2.5 ml) addition of distilled water (2 ml) and 0.1% $FeCl_3$ (0.5 ml) was done and then absorbance was documented at 700 nm. In the blank solution plants extracts were excluded. Ascorbic acid (reference standard) was considered for comparison. The inhibition was recorded using above given formula.

Total anti-oxidant capacity (TAC) (Phospho-molybdate assay)

The phosphomolybdate method was performed to asses an anti-oxidant capacity of compounds. 1 ml of assay mixture comprising of sulfuric acid (0.6 M), sodium phosphate (0.028 M) and ammonium molybdate (0.004 M) were added to sample tubes containing 100 µl of plant extracts (0.2-1.0 mg/ml). In above mixture, plant samples were replaced with methanol and it was used as blank sample. Each mixture was incubated for 90 min. in a hot water bath (95°C). After cooled, samples were subjected to measurement of absorbance at 765 nm. Each of the anti-oxidant procedure given above was performed in triplicates. The above given formula was used to determine the percentage inhibition. Similar procedure was followed for combination of plant extracts (1:1).

Statistical analysis

Analysis of the data was performed using statistical methodology and tools. The results are represented as mean and standard deviation. One-way ANOVA (analysis of variance) was used for comparative analysis. The differences across experimental sets and multiple comparisons were made after that. When p value is lesser than 0.05, differences were found to be statistically significant and when p value is lesser than 0.001, they were interpreted statistically very significant.

Results and Discussion

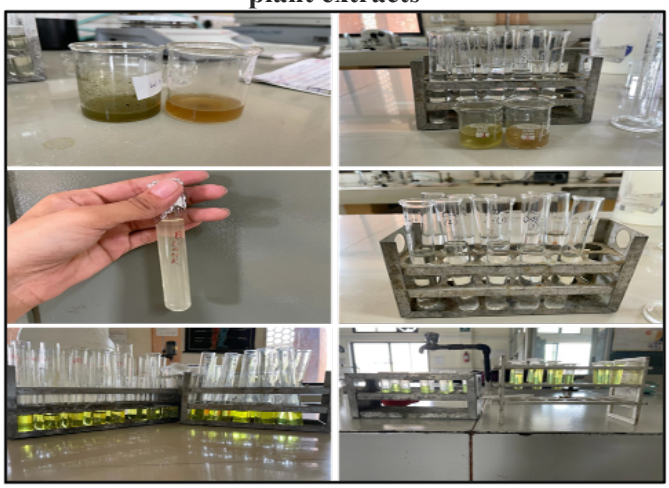
Authentication of plant and its extractive value

Fresh plants were successfully authentication by BSI vide their certificate number BSI/WRC100-1/ TECH/2019/62 dated 19th Dec.2019 and confirmed that, the submitted plant species are *Sansevieria cylindrica* Bojer ex Hook. and *Plumeria obtusa* L. belonging to family of Asparagaceae and Apocynaceae, respectively. We have also evaluated various pharmacognostic parameters of both these plants extracts and results are published (25,26).

Anti-inflammatory activity

This experiment aimed to assess whether hydro-alcoholic extracts of *Sansevieria cylindrica* and *Plumeria obtusa* plants were able to prevent protein denaturation and damage to the RBC membrane. Figure 1 shows the in-process anti-inflammatory activity using plant extracts.

Figure 1. In-process anti-inflammatory study using plant extracts



Anti-inflammatory study of plant extract 1 (PE1; *Sansevieria cylindrica* hydro-alcoholic extract), plant extract 2 (PE2; *Plumeria obtusa* hydro-alcoholic extract) and their combination were assessed against denaturation of BSA. The highest % inhibition level was perceived in hydro-alcoholic extract of (PE1 + PE2) which was 85.33% at a concentration of 1 mg/ml. Statistically, highly significant difference ($p < 0.001$) between combination of plant extracts and standard Ibuprofen was reported. The PE1 and PE2 also exhibited anti-inflammatory activity but was lower compared to standard Ibuprofen in statistically significant manner ($p < 0.05$).

The RBC membranes were effectively stabilized with treatment of plant extracts. The extracts were also significantly inhibited hypotonicity induced lysis and heat-induced hemolysis at various concentrations. The highest membrane stabilization activity was demonstrated by the combination of plants extract (PE1 + PE2) with percentage inhibition of hypotonicity induced lysis of 68.66% which was higher as compared to diclofenac and % inhibition of heat induced lysis of 77.88%. However, the difference of percentage inhibition between the standard and plants extract in combinations was non-significant in both cases. The individual plant extracts also reported good amount of membrane stabilization in non-significant manner. All the plant extracts exhibited the membrane stabilizing activity indicating their added efficacy to their anti-inflammatory potential. The biologically active compounds such as flavonoids, phenolic components, tannins are, may be responsible for an anti-inflammatory and membrane stabilizing effect. The results are supported by published literature. For example, Reshma *et.al.* found *Aegle marmelos* as a potent anti-inflammatory plant with 95.64% inhibition of protein denaturation in water extract whereas, methanolic extract of *Ficus virens* and *Calotropis gigantea* are effective with maximum inhibition of 64% and 90.58% of hemolysis by heat induced and hypotonicity induced methods, respectively (27,28,29).

When a live tissue is getting injured then it leads to inflammation. The metabolism of arachidonic acid plays a significant part in a number of events that make up the mechanisms of inflammation. It is converted through 5-lipoxygenase (5-LOX) pathway to eicosanoids and leukotrienes (LT's), which are known to serve like chemical mediators in wide range of inflammatory process, as compared to cyclooxygenase (COX) pathway, which produces prostaglandins and thromboxane A2 (30). *In-vitro* tests and *in-vivo* assessment of inflammation have been employed in studies to try and recognize medicinal plants with anti-inflammatory potential. However, employing animals in first stages of medication discovery for inflammatory disorders raises ethical concern (31). Inflammatory diseases have been associated with protein denaturation. The suppression of hypotonicity and heat induced lysis of red blood cell membrane will be regarded as a measure of the mechanism of anti-inflammatory study because human red blood cell membranes are comparable to lysosomal membrane (32). All these assays are cheap options for testing the anti-inflammatory activity of herbal medicine. Thus, *in-vitro* studies in beginning are helpful in developing and understanding the mechanism of anti-inflammatory effect of herbal constituents. The results were documented in Table 1 and Figure 2.

Anti-oxidant activity

In this work, *Sansevieria cylindrica* and *Plumeria obtusa* plant extracts were assessed to know their anti-oxidant potential through free radical scavenging effect. Figure 3 reflect the in-process free radical scavenging activity.

DPPH and Phosphomolybdate radical scavenging activity

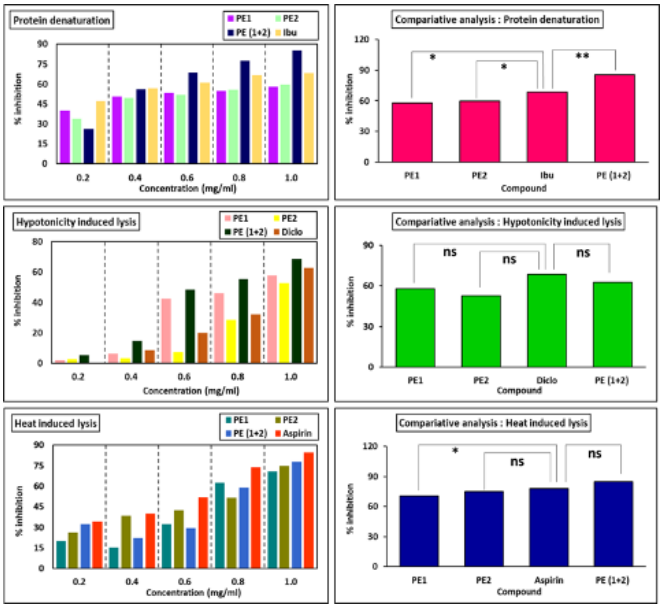
The DPPH radical scavenging effect of PE1, PE2 and the combination of (PE1+ PE2) was evaluated and compared with Ascorbic acid reference standard. Both plant extracts and their combination exhibited radical scavenging effect in concentration-dependent manner which was found to be enhanced with increase in concentration of radical scavenging agent. The PE2

Table 1. The observations for anti-inflammatory activities (n=3)

Concen. (mg/ml)	% inhibition of inflammation± SD											
	Protein denaturation				Hypotonicity induced lysis				Heat induced lysis			
	PE1	PE2	PE 1+2	Ibu.	PE1	PE2	PE 1+2	Diclo.	PE1	PE2	PE 1+2	Asp.
0.2	40.00 (1.73)	34.00 (8.19)	26.33 (5.03)	47.33 (5.51)	1.91 (2.12)	2.63 (1.83)	5.48 (2.27)	0.04 (0.47)	20.11 (11.60)	26.15 (9.40)	32.37 (2.47)	34.30 (7.13)
0.4	50.67 (2.52)	49.67 (10.97)	56.00 (2.00)	56.67 (2.31)	6.18 (4.26)	3.37 (1.66)	14.69 (2.53)	8.64 (6.38)	15.11 (9.65)	38.37 (11.79)	22.16 (2.70)	39.82 (4.64)
0.6	53.33 (4.04)	51.67 (6.51)	68.67 (1.53)	61.00 (1.00)	42.52 (4.51)	7.18 (1.94)	48.59 (2.27)	19.83 (1.52)	32.41 (4.54)	42.62 (2.57)	29.35 (11.13)	51.91 (2.75)
0.8	55.00 (5.00)	55.67 (4.04)	77.67 (1.53)	66.67 (0.58)	46.00 (7.00)	28.66 (3.70)	55.34 (3.55)	32.08 (2.76)	62.37 (4.89)	51.47 (0.84)	58.69 (5.72)	73.72 (1.25)
1.0	58.00 (1.00)	59.67 (1.53)	85.33 (3.51)	68.33 (1.15)	57.78 (11.62)	52.66 (3.93)	68.66 (3.11)	62.63 (5.49)	70.65 (4.00)	74.90 (4.10)	77.88 (7.06)	84.76 (3.35)

Concen: Concentration; PE1: Sansevieria cylindrica extract; PE2: Plumeria obtusa extract; Ibu: Ibuprofen; Diclo: Diclofenac; Asp: Aspirin

Figure 2: The percentage inhibition of protein denaturation and cell lysis



*PE1: Sansevieria cylindrica extract. PE2: Plumeria obtusa extract. Ibu: Ibuprofen. Diclo: Diclofenac. Results are shown as mean ± SD (n = 3). Statistical analysis was performed using GraphPad Prism software; version 6.0 by one-way ANOVA test followed by Tukey's multiple comparison test between both plants extracts separately and in combination vs standard at higher concentration of 1.0 mg/ml. * $p < 0.05$ vs standard. ** $p < 0.001$ vs standard. Not significant (ns) $p > 0.05$ vs standard.*

extract exhibited a good free radical scavenging effect in case of DPPH inhibition at concentration of 1 mg/ml which was statistically higher ($p < 0.001$) than Ascorbic acid at same concentration. The PE1 extract also exhibited free radical scavenging effect, but was seen to be lower in comparison to reference standard and PE2 extract. The radical scavenging activity of combination of extracts (PE1+PE2) was comparable to that of reference standard in non-significant manner ($p > 0.05$). The radical scavenging effect of extracts and reference standard ascorbic acid on DPPH radical decreased in following order: PE2 > Ascorbic acid > (PE 1+PE 2) > PE1.

Figure 3: In-process free radical scavenging activity

In phosphomolybdate assay all the plant extracts exhibited potential anti-oxidant activity. Among the plant extracts, PE1 exhibited 87.20% anti-oxidant which was higher as compared to the standard ascorbic acid. The plant extracts possessed anti-oxidant activity in a concentration-dependent manner (0.2-1 mg/ml) and for the plant extracts PE1, PE1+PE2 the activity was higher than ascorbic acid and for PE2 the activity was comparable to ascorbic acid. Non-significant ($p > 0.05$) difference has been reported for comparison between standard, and all plants extract individually and in combination. Table 2 and Figure 4 display the free radical scavenging by both assay methods.

H_2O_2, NO and Reducing ability assays

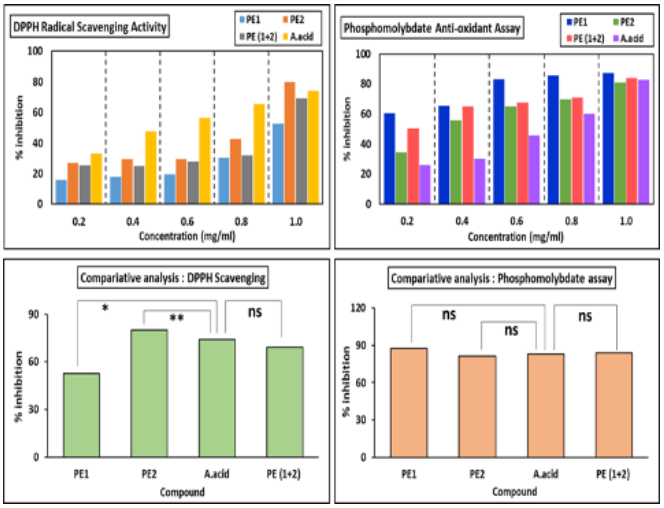
The existence of phenolic group in hydro-alcoholic extract of both plants effectively scavenged the hydrogen peroxide due to the acceptance of its free electrons by the phenolic group of plant extracts and hence neutralized it into water. The H_2O_2 radical scavenging activity of PE1, PE2 and the combination (PE1+PE2) were evaluated and compared with reference standard. Both plant extracts and their combination exhibited radical scavenging effect in a concentration dependent manner (0.2-1 mg/ml) which was seen to raise with raising in concentration of radical scavenging agent. The combination of (PE1+PE2) extract exhibited a free radical scavenging effect for

Table 2: The observations for DPPH and Phosphomolybdate assay (n=3)

Concen. (mg/ml)	Free radical scavenging ± SD							
	DPPH assay				Phosphomolybdate assay			
	PE1	PE2	PE 1+2	A. acid	PE1	PE2	PE 1+2	A. acid
0.2	15.90 (2.15)	26.96 (3.26)	25.26 (1.99)	33.04 (2.39)	60.27 (3.65)	34.38 (9.41)	50.15 (2.01)	26.04 (1.36)
0.4	17.84 (0.97)	29.28 (0.82)	24.86 (1.61)	47.56 (2.77)	65.48 (2.54)	55.80 (3.57)	64.88 (2.01)	30.36 (1.18)
0.6	19.54 (3.24)	29.55 (3.16)	27.85 (7.26)	56.28 (1.42)	83.18 (2.46)	64.88 (1.12)	67.56 (0.93)	45.83 (3.29)
0.8	30.15 (1.78)	42.70 (3.28)	31.88 (5.35)	65.40 (2.46)	85.42 (0.68)	69.49 (1.86)	70.83 (1.44)	59.82 (1.61)
1.0	52.63 (7.13)	79.89 (6.76)	69.12 (1.43)	74.04 (2.00)	87.20 (1.12)	81.10 (0.68)	83.78 (2.46)	82.74 (2.01)

Concen: Concentration; PE1: Sansevieria cylindrica extract; PE2: Plumeria obtusa extract; A. acid: Ascorbic acid

Figure 4: DPPH and Phosphomolybdate scavenging activity of plants extract



*PE1: Sansevieria cylindrica extract. PE2: Plumeria obtusa extract. A. acid: Ascorbic acid. Results are shown as mean ± SD (n = 3). Statistical analysis was performed using GraphPad Prism software; version 6.0 by one-way ANOVA test followed by Tukey's multiple comparison test between both plants extracts separately and in combination vs standard at higher concentration of 1.0 mg/ml. * $p < 0.05$ vs standard. ** $p < 0.001$ vs standard. Not significant (ns) $p > 0.05$ vs standard.*

H_2O_2 at concentration of 1 mg/ml which was 80.56% whereas BHA reported 86.90% free radical scavenging but the comparison was non-significant. ($p > 0.05$). The PE1 extract also exhibited free radical scavenging effect but was lower in comparison to reference standard and PE2 extract.

The NO scavenging activity for the combination of PE1 and PE2 observed was 31.63%, 56.20%, 73.94%, 89.97%, 92.95% at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml, respectively. The more prominent reduction of NO was exhibited by the combination at 1.0 mg/ml which was comparable to the standard ascorbic acid but in non-significant manner ($p > 0.05$). The production of nitrite after incubation of sodium nitroprusside solution in phosphate buffer saline for 40 min. at 37°C was also found to reduce by PE1 and PE2 significantly ($p < 0.05$) when compared with reference standard.

The reducing power assay estimated the anti-oxidant activity by measuring increase in an absorbance due to formation of ferrous ions. In this method, the Fe^{3+} (ferricyanide) from the reagent are converted to ferrous ions because of electron donating ability of plant extracts; which results in increase absorbance (33). The reducing ability of the PE1, PE2 and their combination measured for the concentration upto 1 mg/ml showed an elevation in effect with increased concentration. The combination of PE1 and PE2 extracts were possessed the reducing capacity of 85.90% which was non-significantly ($p > 0.05$) comparable to the standard BHA which was 89.50%. The results of all three assay are given in Table 3 and Figure 5. The results are supported by published literature. Anti-oxidant potential of *Calotropis gigantea* and *Manilkara hexandra* was reported by Iqbal et al. and Dutta *et.al.*, respectively. They have reported that, both plants are good source of an anti-oxidant agent (34, 35).

The DPPH test indicates, the potential of component to be act as a free radical scavenger. The method is dependent on ability of natural anti-oxidant to scavenge protonated free radical i.e. DPPH. The occurrence of odd electron in DPPH exhibits an absorption-spectra at 517 nm when estimated using visible spectroscopy. When any free radical scavenging agent gets paired with this odd electron in DPPH, the absorption observed at 517 nm disappears causing the decolourization, which is stoichiometric and depends on the number of electrons taken by free-radical scavenger (24). Phosphomolybdate assay method is based upon an ability of an anti-oxidant compound to transfer electron to complex and reduce it to form green phosphate complex (36). Hydrogen peroxide is rapidly converted into oxygen and water leads to formation of free radical hydrogen peroxide which can cause lipid peroxidation damaging the DNA (37). NO being a free radical it is responsible for various toxic physiological and pathological events (38). Thus, inhibition or scavenging of all such free radicals might be helpful in avoiding any toxic effect.

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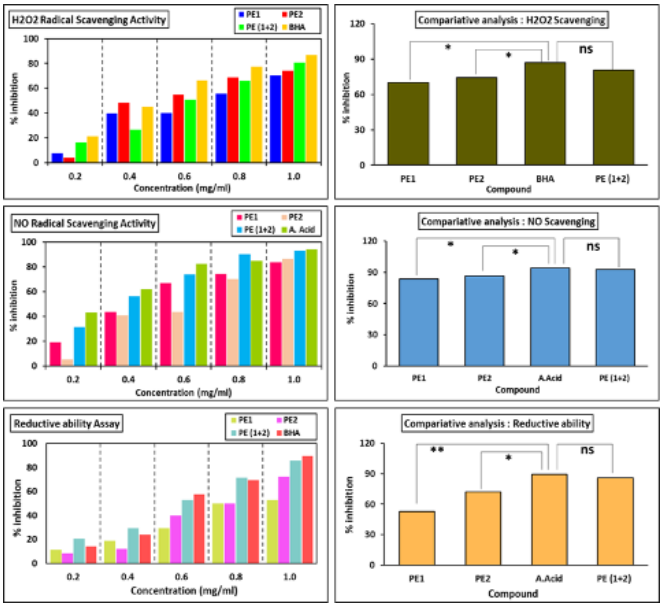
Table 3: The observations of H_2O_2, NO and Reducing ability assay (n=3)

Concen. (mg/ml)	Free radical scavenging ± SD											
	H_2O_2 assay				NO assay				Reducing ability assay			
	PE1	PE2	PE 1+2	BHA	PE1	PE2	PE 1+2	A. acid	PE1	PE2	PE 1+2	BHA
0.2	7.54 (3.64)	3.97 (2.48)	16.27 (3.64)	21.03 (15.31)	19.19 (7.66)	5.26 (3.37)	31.63 (9.69)	43.19 (7.67)	11.17 (4.82)	8.17 (3.18)	20.57 (4.72)	14.07 (5.65)
0.4	39.68 (11.56)	48.41 (4.18)	26.59 (6.11)	44.84 (8.94)	43.45 (6.00)	40.82 (5.42)	56.20 (7.55)	61.98 (7.36)	18.95 (1.59)	12.20 (1.53)	29.13 (4.54)	23.65 (5.73)
0.6	40.08 (1.37)	54.76 (3.57)	50.79 (5.37)	66.27 (0.69)	66.97 (0.59)	43.71 (3.43)	73.94 (3.34)	82.30 (0.27)	29.27 (2.95)	39.80 (3.35)	53.02 (2.81)	57.58 (1.68)
0.8	55.56 (7.75)	68.65 (8.45)	65.87 (8.94)	77.38 (5.95)	74.11 (1.61)	70.04 (3.42)	89.97 (0.08)	84.84 (0.20)	49.81 (1.47)	49.91 (6.78)	71.58 (1.86)	69.14 (4.72)
1.0	70.24 (6.30)	74.21 (4.81)	80.56 (3.00)	86.90 (2.38)	83.66 (0.50)	86.55 (2.55)	92.95 (3.58)	94.17 (0.27)	52.88 (6.08)	72.15 (3.69)	85.90 (2.39)	89.50 (2.27)

Concen: Concentration; PE1: Sansevieria cylindrica extract; PE2: Plumeria obtusa extract; BHA: Butylated hydroxyanisole; A. acid: Ascorbic acid

Figure 5. H_2O_2, NO and Reducing ability anti-oxidant assay of plants extract



*PE1: Sansevieria cylindrica extract. PE2: Plumeria obtusa extract. BHA: Butylated Hyroxyanisole. A. acid: Ascorbic acid. Results are shown as mean ± SD (n = 3). Statistical analysis was performed using GraphPad Prism software; version 6.0 by one-way ANOVA test followed by Tukey's multiple comparison test between both plants extracts separately and in combination vs standard at higher concentration of 1.0 mg/ml. * $p < 0.05$ vs standard. ** $p < 0.001$ vs standard. Not significant (ns) $p > 0.05$ vs standard.*

Conclusion

The hydro-alcoholic extracts of *Sansevieria cylindrica* and *Plumeria obtusa* and their combination demonstrated significant anti-oxidant and anti-inflammatory activities which were evident from the various assays carried out in this study. The existence of significant amount of phenolic content in hydro-alcoholic extracts of *Sansevieria cylindrica* and *Plumeria obtusa* and their combination might be responsible for these activities. The results revealed that, plants extract reported, notable anti-oxidant and anti-inflammatory activities. In many instances, it was either comparable or greater than reference standards used in this research work. However, further *in-vivo* studies could be helpful to fully elucidate anti-inflammatory and anti-oxidant effects of plants and their mode of action, for development of potential herbal drug formulation.

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Conflict of interest

All the authors involved in present study hereby declare, no conflict of interest.

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