VOLUME 8 (SPECIAL ISSUE) 2022

ISSN 2454-3055

Manuscripts under Special issue are published with the Theme
"Modern Perspectives of Biological Sciences"

Guest Editor: Dr. S. Mohanasundaram
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INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 8, Special Issue, 91-96 (2022)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Antioxidant Activity of the Methanolic Leaf Extracts of *Alangium salviifolium*, *Tecoma stans* and *Pisonia alba* by Nitric Oxide (NO) Free Radical Scavenging Assay

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Received: 4th August, 2022; **Accepted:** 20th August, 2022; **Published online:** 15th September, 2022

https://doi.org/10.33745/ijzi.2022.v08i0s.011

Abstract: Extracts of *Alangium salviifolium*, *Tecoma stans* and *Pisonia alba* were examined in methanol to see whether or not they have the ability to scavenge free radicals. The extract was able to considerably cut down on nitric oxide radicals while simultaneously scavenging free radicals. Common antioxidants such as ascorbic acid also possesses comparable antioxidant action, but at lower concentrations. It was discovered that an extract concentration of 200 g/ml had the highest potential to scavenge for free radicals.

Keywords: Antioxidant, Medicinal herbs, DPPH, Nitrate assay, Free radicals, Scavanging

Citation: Sri Ramya T., Swarnalatha K. and Sammaiah G.: Antioxidant activity of the methanolic leaf extracts of *Alangium salviifolium*, *Tecoma stans* and *Pisonia alba* by nitric oxide (NO) free radical scavenging assay. Intern. J. Zool. Invest. 8(Special Issue): 91-96, 2022.
https://doi.org/10.33745/ijzi.2022.v08i0s.011

Introduction

Antioxidant properties of synthetic compounds such as butylated hydroxyanisole, butylated hydroxytoluene, and tertiary butylhydroquinone have been established in the human body in the face of free radical-induced oxidative damage (Issa *et al*., 2006; Kaliora *et al*., 2006). Due to their toxicity and detrimental impact on human health, synthetic antioxidants are rapidly being outlawed (Sasaki *et al*., 2002; Bajpai *et al*., 2014). There are several compounds found in medicinal plants that have anti-inflammatory, anti-bacterial, and anticancer activities (Hernandez *et al*., 2000; Polya, 2003; Maruthanila *et al*., 2014). For thousands of years, herbal medicine has been utilised to treat infectious illnesses and will continue to do so in the future (Cragg and Newman, 2007). People who consume more plant-based meals are less likely to die from sickness, according to previous studies (Chidambaram *et al*., 2013). Antioxidant properties of natural origin plants have lately attracted much attention because of their improved safety and market

appeal (Gorinstein *et al*., 2003). Furthermore, natural ingredients make up almost 60% of commercially available anti-tumor and anti-infective drugs (Shridhar *et al*., 2009).

Phytochemicals from plants have been shown to provide health advantages or to be useful in the treatment of disease. Only 10% of the possible secondary metabolites produced by plants have been identified. Antibacterial, anti-inflammatory, antihypertensive, anti-diabetic, and a host of other health benefits are provided by these metabolites (Mallikarjun *et al*., 2007; Ayoola *et al*., 2008,). Examining elements in pharmaceutical and health care products is important since they have a beneficial influence on human health. Anemia, arthritis, inflammation, neurodegeneration, and Parkinson's disease are just a few of the disorders that antioxidants help to prevent by binding to free radicals in the body (Oke and Hamburger, 2002; Odakoya *et al*., 2006). Hence, there is a need to investigate plant species' antioxidant capacity. The medicinal herbs *Alangium salviifolium*, *Tecoma stans*, and *Pisonia alba* were tested for their antioxidant capabilities in this study.

Flowering plant of the Cornaceae family, Alangiumsalviifolium is also known as sage-leaved alangium. In Tamil, it is referred to as Alanji. It is utilised in Ayurvedic medicine for the treatment of rheumatism and haemorrhoids by use of its roots and fruits. Analgesic, anthelmintic, diarrhoeal and hypoglycemia are only a few of the traditional uses for this herb. The trumpet vine family, Bignoniaceae, includes the perennial shrub *Tecoma stans*, which blooms only in the Americas. A variety of common plant names include yellow bells, yellow elder, and ginger-thomas. Alkaloids, phytosterols, quinine and amino acids are just a few of the chemical compounds found in this plant. There are also glycosides, flavonoids and saponins to name a few. In traditional folk medicine, roots are reported to have diuretic, tonic, anti-syphilitic, and vermifuge properties. Stomach aches are treated with a flower and bark infusion in Veracruz. A flowering plant genus, *Pisonia alba* belongs to the Nyctaginaceae family. Leaves may be fed to cattle and are often used to treat arthritis and rheumatoid diseases.

Thus, these above mentioned plants were employed in the study. Blood pressure, neuronal signal transduction, platelet function, antibacterial and anticancer properties are only a few of the many functions of nitric oxide (Jagetia *et al*., 2004). After interacting with oxygen and superoxide radicals, NO becomes toxic. A large amount of cell damage may be caused by the reaction products (Vriesman *et al*., 1997). As a kind of natural medicine, plants have long been used to cure a wide range of diseases.

Materials and Methods

Collection and processing of specimens:

All the plant *Alangium salviifolium, Tecoma stans and Pisonia alba* leaves were collected from Warangal area, India. It was stored in a clean, dry area. Tap water was used to properly clean the plants' leaves, and only distilled water was used to finish the process. The dried and powdered leaves were used.

Extraction:

Soxhlet was used to extract *A. salviifolium*, *Tecoma stans*, and *Pisonia alba* dry powders using 95 per cent methanol (Merck) in each case. A week of soxhlation with methanol was necessary to get the extract. Extract was then evaporated in a water bath at 50°C to provide crude for the antioxidant test (Gupta *et al*., 2011).

Neutralization of reactive oxygen species:

A spectrophotometer can detect the Nitrite ions in aqueous solution at 550 nm by detecting the Nitrite oxide formed when sodium nitroprusside in aqueous solution with physiological pH is treated with Griess reagent (Kumar *et al*., 2008).

Procedure:

Distilled water was used to dissolve the plant extract. In standard phosphate buffer saline (0.138 M, pH 7.4), Sodium Nitroprusside (5 mM) has been treated for 3 h at 29°C using different methanol extract concentrations (100-500 g/ml).

Table 1: The optical density value of the methanolic leaves extracts of *A. salviifolium, T. stans and P. alba* (NO Assay)

Conc. µg/ml	*A. salviifolium*	*T. stans*	*P. alba*	Standard
	Optical Density (OD) at 550 nm			
Blank	0.6157	0.6157	0.6157	0.6157
100	0.4843	0.4921	0.4746	0.4618
200	0.3193	0.3024	0.3237	0.2841
300	0.3507	0.3713	0.3619	0.3397
400	0.3848	0.3911	0.3801	0.3728
500	0.3964	0.4182	0.4014	0.4482

Table 2: Inhibition efficiency of methanolic leaves extracts of *A. salviifolium, T. stans* and *P. Alba* (NO Assay)

Conc. µg/ml	*A. salviifolium*	*T. stans*	*P. alba*	Standard
	Inhibition (%)			
100	21.342	20.075	22.917	24.996
200	48.140	50.885	47.426	53.857
300	43.040	39.695	41.221	44.827
400	37.502	36.479	38.265	39.451
500	35.618	32.077	34.806	36.205

Experimentation with no test chemicals but with the same quantity of buffer was performed in the same manner as that of the control experiment. Samples were diluted with 1 ml of Griess reagents after 3 h of incubation. When Nitrite was diazotized with sulphanilamide and then coupled with napthylethylenediamine hydrochloride, the absorbance of the colour generated was measured at 550 nm using a spectrophotometer. In the same way as with methanol extract, ascorbic acid was used. The percentage of inhibition was calculated using following formula as compared to the standard.

Per cent inhibition= [O.D. of control - O.D. of Test/O.D. of control] X 100

Results and Discussion

Smooth muscle relaxation, neuronal signalling, platelet aggregation inhibition, and cell-mediated toxicity control are only few of the physiological processes that NO suppresses (Triathi *et al*., 2007). Inflammation, cancer, and other diseases have been linked to nitric oxide in addition to reactive oxygen species (Bhattacharya, 2015). NO is a tissue- and organ-specific free radical moiety thought to be critical in neuromodulation.

Phytochemical studies on *A. salviifolium*, *T. stans*, and *P. alba* showed a variety of compounds in addition to triterpenoids and essential oils, some of which are especially significant for antioxidative action. Free radical scavenging properties were revealed in this DPPH study. *A. salviifolium*, *T. stans*, and *P. alba* methanolic leaf extracts were analysed for their optical density and inhibitory value (Tables 1, 2). These extracts, together with ascorbic acid were able to neutralise free radicals such as NO and nitric oxide (Figs. 1-3) (Gupta *et al*., 2011). Methanol extracts from all the trousers exhibited maximum activity of 48.14%, 50.88% and 47.42%, respectively, whereas ascorbic acid inhibited the enzyme by 53.87 per cent at 200 g/ml (the concentration used in this experiment). They found that many bioactive compounds with antioxidant potential were present in the methanol extracts of *A. salviifolium*, *T. stans* and *P. alba* plants (Kumar *et al*., 2008). Oxidative stress-related disorders may benefit from the plant extract's ability to prevent and delay their development. Natural antioxidants may be able to take advantage of this. Compared to ascorbic acid, methanol extracts of the following plants showed substantial antioxidant activity: *A. salviifolium*, *T. stans*, and *P. alba*. Natural

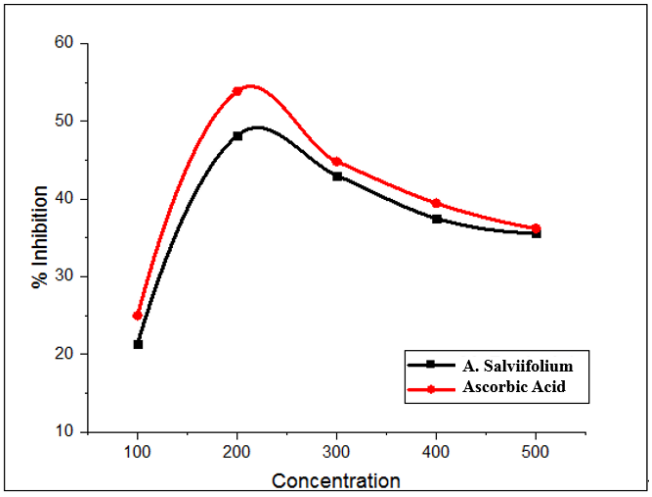

Fig. 1: Nitric oxide scavenging assay of *A. Salviifolium* and ascorbic acid.

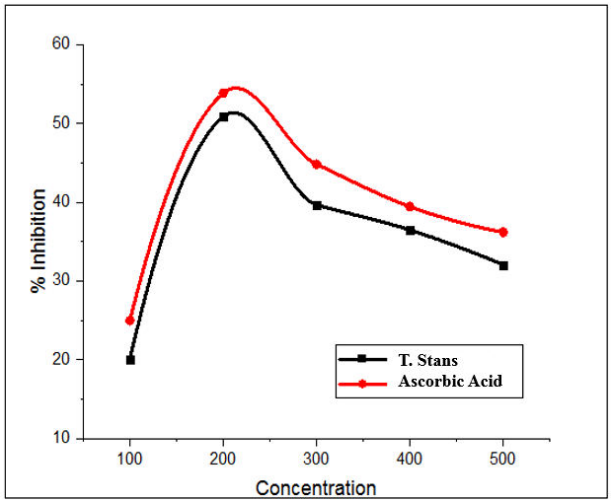

Fig. 2*:* Nitric oxide scavenging assay of *T. Stans* and ascorbic acid.

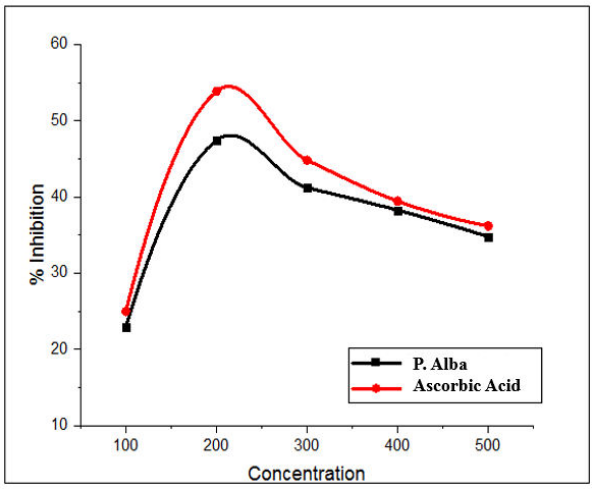

Fig. 3: Nitric oxide scavenging assay of *P. Alba* and ascorbic acid.

antioxidants from *A. salviifolium*, *T. stans*, and *P. alba* may be useful in preventing or reducing the progression of oxidative stress-related degenerative disorders, according to this study. Researchers and pharmaceutical companies may be able to use these findings to develop new drugs to treat diseases caused by oxidative stress.

Conclusion

The antioxidant activities of *A. salviifolium*, *T. stans*, and *P. alba* extracts were examined in this study. This research suggested that they may have anti-inflammatory effects. High phenolic antioxidant components have been found in this investigation. *T. stans*, *P. alba*, and *A. salviifolium* have now been added to the growing body of data pointing to their usage as possible traditional medicines in this research. It's possible that these new findings will be effective in the battle against degenerative illnesses including cancer, cardiovascular disease, and neurodegenerative disease, as well as adding to the increasing database of medicinal plants. As a consequence, the identification of novel lead compounds that combat diseases caused by free radicals may be aided by the isolation of more active principles.

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