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Preliminary screening of *anaphyllum wightii* schott tubers for anti-inflammatory and antioxidant activity.

Dharsana JN[1]*, Sr. Molly Mathew[2], Premkumar N[3], Diljit S Kuttoor[4]

1.Department of Pharmaceutical Chemistry, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur-670503, Kerala, India.

2.Malik Deenar College of Pharmacy, Kasargode, Kerala.

3.Department of Pharmacy Practice, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur-670503, Kerala, India.

4.Department of Pharmacology, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur-670503, Kerala, India.

ABSTRACT

To evaluate the preliminary phytochemical, antioxidant and anti-inflammatory activity of77 *Anaphyllum wightii* Schott. Bovine Serum Albumin denaturation inhibition assay was used for the evaluation of in-vitro anti-inflammatory activity and DPPH. Free radical scavenging and super oxide scavenging assays were used to assess the antioxidant activity of chloroform and aqueous extracts of the tubers of *Anaphyllum wightii* Schott**.** In anti-denaturation study, it was observed that chloroform extract showed greater percentage of inhibition of bovine serum albumin denaturation ie 49.2% whereas aqueous extract showed 38.4% at the 400µg/ml concentration respectively. In case of antioxidant screening also, chloroform extract showed better antioxidant power compared to aqueous extract dose dependently. Among the two extracts evaluated for anti-inflammatory and antioxidant activities chloroform extract of *Anaphyllum wightii* was found to possess significantly good anti-inflammatory and antioxidant activities and this can be attributed to the presence of alkaloids, saponins, flavonoids and phenolic compounds.

Keywords: *Anaphyllum wightii*, Anti-inflammatory, Antioxidant, DPPH.

*Corresponding Author Email: dharsanaapsc@gmail.com

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INTRODUCTION

Plant dugs have been a major source for the treatment of diseases for a long time. They have been used in the traditional medicine on the basis of experiences and practice. With the advent of modern systems of medicine need has been felt to investigate the active constituents present in these plants. Various molecules have been isolated, characterized and tested for their related pharmacological activities.

When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form of stress. The response tothe stress of tissue damage is called as inflammation. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Whether loss of function occurs depends on the site and extent of injury. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion[1].

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. For example, enzymes lose their activity, because the substrates can no longer bind to the active site[2].

Oxidative stress is induced by wide range of environmental factors including UV stress, pathogen invasion (hypersensitive reaction), herbicide action and oxygen shortage. Oxygen deprivation stress in plant cells is distinguished by three physiologically different states: transient hypoxia, anoxia and re-oxygenation. Generation of reactive oxygen species (ROS) is the characteristic feature for hypoxia and essentiallyfor re-oxygenation. Of the ROS, hydrogen peroxide (H_2O_2) and superoxide ($O^{2.-}$) are both produced in number of cell reactions, including iron catalyzed Fenton-reaction and by various enzymes such as lipoxygenases, peroxidases, NADPH oxidase and xanthine oxidase. The main cellular components susceptible to damage by free radicals are lipids (peroxidation of unsaturated fatty acids in cell membrane), proteins (denaturation), carbohydrates and nucleic acids[3].

Both inflammation and free radical damage are inter-related aspects that influence each other. As said above proteins are susceptible to undergo denaturation by formation of free radicals and the mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species from activated neutrophil and macrophages. This over-production leads to tissue injury by damaging the

macromolecule and lipid peroxidation of membranes. In addition, ROS propagate inflammation by stimulating the release of the cytokines such as interleukin-I, tumor necrosis factor-α, and interferon-γ, which stimulate recruitment of additional neutrophil and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation[4,5]. Thus our study aims to find a natural remedy that will be useful to treat both inflammation and free radical damage.

MATERIALS AND METHODS

Collection and Authentication

Genus – *Anaphyllum beddomei* Engl. and *Anaphyllum wightii* Schott were reported from the high ranges of Western Ghats.The two species in this genus are similar in appearance to those in the genus Anaphyllopsis. Genus *Anaphyllum wightii* is listed as an endemic and threatened genus of South India[6]. The species of the genus Anaphyllum are found in marshes. They are characterized by pinnate leaves and twisted spathe for the spadix. Anaphyllum is a genus of flowering plants in the Araceae family. The tribal communities (Kani Tribes, Malasars, Kadars, Pulayars, Madhuvars etc) use these plants as food and as an antidote to snake bite. Leaves of *Anaphyllum beddomei* form a part of tribal diet. Arun et al. reported the use of the corms of *Anaphyllum wightii* (Keerikizhangu), as an antidote to snake bite along with some medicinal plants[7].

Plant material

Plant tubers were collected from Wynad hills, Wynad district, Kerala, India. The taxonomical identification of the plant was done by Dr. N. Sasidharan, Scientist-F, Programme co-ordinator, FE & BC division, Kerala Forest Research Institute, Peechi, Trissur. The voucher specimen was preserved in Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala.

Preparation of plant extract

The collected plant tubers was dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh. About 200g of powdered materials were extracted with chloroform and water. The extracts are then concentrated using vacuum evaporator and dried under reduced pressure.

Chemicals

Bovine serum albumin (BSA) 5%, phosphate buffer saline (pH 6.3), EDTA, Nitro blue tetrazolium (NBT), Riboflavin, DPPH (2,2-diphenyl-1-picrylhydrazyl) and Indomethacin were purchased from Merck Pharmaceuticals.

Phytochemical screening

The concentrated extracts were used for preliminary screening of various phytoconstituents viz, carbohydrates, amino acid, alkaloids, tannins and flavonoids were detected by usual methods prescribed in standard tests[8,9].

In-vitro Bovine serum albumin denaturation inhibition assay[10]

The reaction mixture 3ml contained, 50 µl of the test solution (100, 200, 400µg/ml was prepared in methanol). 450µl of 5% w/v BSA was added to all the above test tubes. For control tests, 50 µl of distilled water instead of test solution. The test tubes were incubated at 37ºC for 20 minute and then heated at 57ºC for 3 minutes. After cooling the test tubes, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. The absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660nm.

% Protein denaturation inhibition = [(Absorbance of control – Absorbance of test)/Absorbance of control] × 100

DPPH Free Radical Scavenging Assay[11,12,13]

1 ml of the test compound in various concentrations (50, 100,150, 200 & 250 µg/ml was added to 1ml of 0.1 mm solution of DPPH in methanol. After 30 minutes, absorbance was measured at 517 nm, using a spectrophotometer (SHIMADZU, UV 1800). A 0.1 mM solution of DPPH in methanol was used as blank, whereas ascorbic acid was used as a reference standard. All tests were performed in triplicate. Percent inhibition was calculated using equation,

Percent inhibition = [(Control ×Test)/Control]×100

Superoxide Scavenging Assay[11,12,13]

1ml of the test compound in various concentrations (50, 100, 150, 200 & 250 µg/ml) was added to 0.2ml of EDTA, 0.1ml of NBT, 0.05 ml of riboflavin and 2.6 ml phosphate buffer. The control tubes were also set up where DMSO was added instead of the plant extracts. All the tubes were vortexed and the initial optical density was measured at 560 nm in a spectrophotometer. The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560 nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity. All tests were performed in triplicate. Percent inhibition was calculated using equation,

Percent inhibition = [(Control × Test)/Control]×100

RESULTS AND DISCUSSION

Phytochemical studies

From the phytochemical study, it has evaluated the presence of alkaloids, flavonoids, phenols, and saponins. (Table 1)

Table 1. Preliminary phytochemical screening of the chloroform and water extracts of tuber of *Anaphyllum wightii*.

Chemical constituents	Test	Chloroform	Water
Alkaloid	Dragendroff's reagent	-	-
	Mayer's reagent	+	+
	Wagner's reagent	+	+
	Hager's reagent	+	+
Amino acid	Millon's test	-	-
Proteins	Lowry's method	-	-
Phenols	Folin test	+	+
Fats	Filter paper test	-	-
Carbohydrates	Molish test	-	-
	Barfoed's test	-	-
Starch	Iodine test	-	-
Saponins	Form test	+	+
Sugar	Anthrone Reagent Test	-	-
Steroids	Liebermann-Burchard test	+	+
Flavanoids	Ethyl acetate test	+	-
Cardiac glycosides	Ferric chloride test	-	+
Quinone	Sodium hydroxide test	+	-
Tannins	Feric Chloride Test	+	+

In-vitro anti-inflammatory activity (Bovine serum albumin denaturation inhibition assay)

Both chloroform and aqueous extracts of *Anaphyllum wightii* inhibited the denaturation of Bovine serum albumin (BSA). The degree of inhibition of BSA denaturation increased with the increase in the concentration of both the extracts, that the anti-denaturation of the drug will be more at higher concentration. As shown in Table 2 and 3 among the two extracts under the study, chloroform extract (from 27.5±0.16 % to 49.2±0.53%) has shown better inhibition of BSA denaturation at any concentration compared to aqueous extract (from 22.1±0.28% to38.4±0.13%). The standard drug Indomethacin showed 88.9±0.46% inhibition denaturation at 100µg/ml concentration.

Table 2. Effect of chloroform extract of *Anaphyllum wightii* on in-vitro Bovine serum albumin denaturation inhibition.

Sl. No.	Group	Concentration (µg/ml)	% Inhibition of denaturation
1	Control	-	-
2	Indomethacin	100 µg/ml	88.9±0.46
3	Chloroform	100 µg/ml	27.5±0.16
4	Chloroform	200 µg/ml	33.0±0.57
5	Chloroform	400 µg/ml	49.2±0.53

Values are Mean± SEM, n= 3

The method of anti-denaturation of BSA was chosen to evaluate anti-inflammatory property of *Anaphyllum wightii*. In anti-denaturation assay the denaturation of BSA is induced by heat treatment. The denatured BSA expresses antigens associated to Type III hyper-sensitive reaction which are related to diseases such as serum sickness, glomerulo-nephritis etc[14]. Heat denatured proteins are as effective as native proteins in provoking delayed hypersensitivity[15]. Moreover it was already proved that conventional NSAID's like phenylbutazone and indomethazine does not act only by the inhibition of endogenous prostaglandins production by blocking COX enzyme but also by prevention of denaturation of proteins[16]. Thus anti-denaturation assay is the convenient method to check the anti-inflammatory activity. In our results both the extracts has shown considerable anti-inflammatory activity and chloroform extract was found to be more potent than aqueous extract. The secondary metabolites like phenolic compounds and tannins which were found in preliminary phytochemical screening might be responsible for this activity.

Table 3. Effect of aqueous extract of *Anaphyllum wightii* on in-vitro Bovine serum albumin denaturation inhibition.

Sl. No.	Group	Concentration (µg/ml)	% Inhibition of denaturation
1	Control	-	-
2	Indomethacin	100 µg/ml	88.9±0.46
3	Aqueous	100 µg/ml	22.1±0.28
4	Aqueous	200 µg/ml	29.7±0.32
5	Aqueous	400 µg/ml	38.4±0.13

Values are Mean± SEM,n= 3

Antioxidant activity

Several concentrations ranging from 50-250µg/ml of the chloroform and aqueous extracts of *Anaphyllum wightii* were compared for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the extracts in a concentration dependent manner up to the given concentration in all the models. The absorbance of the two extracts increased with the rise in their concentrations since the absorbance is directly proportional to the antioxidant property. As shown in table 4 and 5 among the two extracts under the study the chloroform extract (from 22.98±2.11% to 49.02±4.11% for DPPH free radical scavenging assay and from 29.48±1.44% to 60.20±3.43% for super oxide scavenging assay) indicates better antioxidant power compared to aqueous extract (from 20.61±1.32 % to 41.71± 4.22 % for DPPH free radical scavenging assay and from 24.48±1.33% to 39.78±3.64% for super oxide scavenging assay) (Table 6 and 7). The standard drug ascorbic acid showed 72.78±3.97% antioxidant power at 50µg/ml concentration.

Table 4. Effect of chloroform extract of *Anaphyllum wightii* on in-vitro antioxidant activity by DPPH radical scavenging assay

Concentration (µg/ml) of Chloroform extract	% Scavenging	Concentration (µg/ml) of Ascorbic acid	% Scavenging
50	22.98±2.11	10	35.63±2.32
100	31.36±2.14	20	48.69±2.43
150	35.64±3.34	30	53.67±2.52
200	42.02±3.17	40	61.91±3.57
250	49.02±4.11	50	72.78±3.97

Values are Mean± SEM,n= 3

Table 5. Effect of aqueous extract of *Anaphyllum wightii* on in-vitro antioxidant activity by DPPH radical scavenging assay

Concentration (µg/ml) of aqueous extract	% Scavenging	Concentration (µg/ml) of Ascorbic acid	% Scavenging
50	20.61±1.32	10	35.63±2.32
100	25.78±2.44	20	48.69±2.43
150	30.02±3.22	30	53.67±2.52
200	36.36±3.74	40	61.91±3.57
250	41.71±4.22	50	72.78±3.97

Values are Mean± SEM, n= 3

Table 6. Effect of chloroform extract of *Anaphyllum wightii* on in-vitro antioxidant activity by superoxide scavenging assay.

Concentration (µg/ml) of Chloroform extract	% Scavenging	Concentration (µg/ml) of Ascorbic acid	% Scavenging
50	29.48±1.44	10	35.63±2.32
100	31.89±1.07	20	48.69±2.43
150	46.03±2.88	30	53.67±2.52
200	56.28±2.73	40	61.91±3.57
250	60.20±3.43	50	72.78±3.97

Values are Mean ± SEM, n=3

Table 7. Effect of aqueous extract of *Anaphyllum wightii* on in-vitro antioxidant activity by superoxide scavenging assay

Concentration (µg/ml) of Aqueous extract	% Scavenging	Concentration (µg/ml) of Ascorbic acid	% Scavenging
50	24.48±1.33	10	35.63±2.32
100	28.34±1.16	20	48.69±2.43
150	31.69±2.22	30	53.67±2.52
200	35.52±2.43	40	61.91±3.57
250	39.78±3.64	50	72.78±3.97

Values are Mean± SEM,n= 3

Free radicals are chemical entities that can exist separately with one or more unpaired electrons[17]. The propagation of free radicals brings about a myriad of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by these free radicals. The oxidative stress exerted due to these free radicals has been implicated in the pathology of various diseases like diabetes, inflammations, cardiovascular complications, cancer and ageing[18]. Antioxidants offer resistance against the oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation and by many other mechanisms and thus prevent disease[19].

In our study, both the extracts has shown considerable scavenging of free radicals in all the *in vitro* models studied. DPPH is a stable free radical. The *in vitro* study carried out on this radical is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH[20]. This radical reacts with suitable reducing agents, the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up[21]. From the present results, it may be concluded that *Anaphyllum wightii* reduce the radical to the corresponding hydrazine when they react with the hydrogen donors in the antioxidant principles. (Table 4,5)

Superoxide dismutase catalysis the dismutation of highly reactive superoxide anion to oxygen and hydrogen peroxide[22]. Superoxide anion is the first reduction product of oxygen which is measured in terms of inhibition of generation of 0_2.[23](Table 6,7)

Preliminary phytochemical screening revealed the presence of alkaloids, phenolic compounds, tannins and flavonoids in *Anaphyllum wightii*. Phenolics, flavonoids and tannins have been proved to be responsible for the antioxidant activity of various medicinal plants reported earlier[24,25,26]. Hence, these may be responsible for the observed activity.

CONCLUSION

Our investigation clearly demonstrates that chloroform and aqueous extracts of *Anaphyllum wightii* possess significant anti-inflammatory and antioxidant activity. Among them chloroform extract was found to be more potent than the aqueous extract. Further studies are recommended to isolate the active principle responsible for these activities.

REFERENCE

1. Gerard J Tortora, Sandra Reynolds. Principles of Anatomy and Physiology. 7th ed., Harper Collins College Publishers; 1993: 695.
2. Denaturation (Biochemistry), Wikipedia the free encyclopedia, available at http://en.wikipedia.org/wiki/denaturation(biochemistry)(Oct2010).

3. Olga blokhina, Eija Virolainen, Kurt V Fagerstedt. Antioxidants, oxidative damage and oxygen deprivation stress: a Review. Annals of Botany 2003; 91: 179-194.
4. Vaidyaratnam P S Varier's. Indian Medicinal Plants: A compendium of 500 species. Volume 1, Kottakkal: Arya Vaidya Sala, Coll. No. AVS 1481;1994.
5. Nadkarni KR, Indian Materia Medica, 2001; 1:114.
6. Ahmedullah M, Nayar MP. Endemic plants of the Indian Region, Vol. I, Penisular India, Flora of India series IV, Botanical Survey of India; 1987; 205-208.
7. Arun V, Liju VB, Reena John JV, Parthipan B, Renuka C. Traditional remedies of Kani Tribes of Kottoor reserve forest, Agasthyaavanam, Thiruvananthapuram, Kerala. Vol 6, 2007: 589-594.
8. Kokate CK. Pharmacognosy. 16th ed., Mumbai: Nirali Prakashan; 2009.
9. Harborne JB. Phytochemical methods, A guide to Modern Techniques of Plant Analysis, 3rd ed., London: Champan& Hall; 1998.
10. Williams LAD, Connar AO, Latore L. The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. West Indian Med J 2008; 57: 327-331.
11. Ansari NM, Houlihan L, Hussain B, Pieroni A. Antioxidant Activity of Five Vegetables Traditionally Consumed by South-Asian Migrants in Bradford, Yorkshire, UK. Phytother Res 2005; 19: 907-911.
12. Molyneux P. The use of stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci and technol 2004; 26: 212-219.
13. Anandjiwala S. Antioxidant activity of stem bark of *Thespepsia populnea*. Journal of Natural Remedies 2007; 7(1): 135-138.
14. Fayyaz Ahmad, Rafeeq A Khan, Shahid Rasheed. Study of analgesic and anti-inflammatory activity from plant extracts of *Lactuca scariola* and *Artemisia absinthium*. J Islam Academic Sci 1992; 5: 111-114.
15. Gell PGH, Benacerraf B. Studies on hypersensitivity-II delayed hypersensitivity to denatured proteins in guinea pig. Immunology 1959; 2: 64.
16. Insel PA. Analgesic-Antipyretics and Anti-inflammatory Agents: Drugs Employed in the Treatment of Rheumatoid Arthritis and Gout. In, Goodman and Gilman's The Pharmacological Basis of Therapeutics. New York: McGraw Hill; 1990; 9: 638 – 681.

17. Jeyaprakash K, Chinnaswami P. Antioxidant property of *Spirulina* and Liv-52 against lead induced toxicity in albino rats. Journal of Natural Remedies 2007; 7(1): 80 - 85.
18. Kolhapure SA, Balaraman R, Bafna P. Antioxidant activity of DHC-1: A herbal formulation. Journal of Ethnopharmacology 2004; 94: 135-143.
19. Lemberkovics E, Czinner E. Comparative evaluation of *Helichrysis flos* herbal extract as dietary sources of plant polyphenols and macro micro elements. Journal of Food Chemistry 2002; 78: 119-125.
20. Mao L, Zheng X, Feique MB. *In-vitro* and *in-vivo* antioxidant activities of Daylily flowers and the involvement of phenolic compounds. Asia Pacific Journal of Clinical Nutrition 2007; 16: 196-205.
21. Kaleem M, Asif MA. Anti-diabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin induced diabetic rats. Journal of Singapore Medicine 2006; 47: 670-676.
22. Ravi Shankar GA, Murthy KN, Giridhar P. Antioxidant property of *Decalepi shamiltonii*. Indian Journal of Experimental Biology 2006; 44: 132-137.
23. Rathod PI, Mello PM. Evaluation of antioxidant activity of anthocyanin's. Indian Drugs 2006; 43: 60-67.
24. Kumar PV, Shashidhara S, Kumar MM, Sridhara BY. Effect of *Luffaechinata* on lipid peroxidation and free radical scavenging activity. Journal of Pharmacy and Pharmacology 2000; 52: 891-898.
25. Velaven S Nagulendran K. *In vitro* antioxidant activity of *Asparagus racemosus* root. Pharmacog Mag 2007; 3: 26-32.
26. Robak J, Grygleweski RJ. Flavonoids are scavengers of superoxide anions. Biochemical Pharmacology 1998; 37: 837-845.