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Antioxidant Activity of Various Extracts of Leaves of *Anacardium Occidentale* (Cashew)

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ABSTRACT

The antioxidant properties and the effect on nitric oxide (NO) production of various extracts of leaves of *Anacardium occidentale* were investigated. Radical-scavenging potential was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Griess assay was used to assess NO-inhibitory activity of the extracts. The antioxidant activity of aqueous, ethanol, and petroleum ether (60-80^{0}C) extracts of the leaves of *Anacardium occidentale* was estimated. The order of the antioxidant potency of the plant extract is ethanol > aqueous > petroleum ether. The results suggest that the leaves of *A.occidentale* are a potent source of natural antioxidants.

Key words: Antioxidant activity, *Anacardium occidentale* extracts, Total Polyphenol Content, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical, Nitric oxide.

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INTRODUCTION

The antioxidants comprise of a range of substances that play a role in protecting biological systems against the deleterious effects of oxidative processes on macromolecules, such as proteins, lipids, carbohydrates and DNA. Many of those substances which contribute to the prevention and treatment of diseases in which reactive species of oxygen are involved, are natural molecules of plants. This protection can be explained by the capacity of the antioxidants of plants to scavenge free radicals [1,2]. The Malaysian population consumes traditional vegetables and herbs, raw or cooked as accompaniments with their main meal. Many of these vegetables are claimed to possess medicinal properties although there are no scientific evidences to support the claims. One of the commonly consumed vegetable is the leaves of *A. occidentale*. *A. occidentale* has been used in the treatment of various diseases including malaria and yellow fever as well as diarrhea [3]. A. occidentale is a tropical tree indigenous to Brazil, and is a member of the family Anacardiaceae. However, now it is also widely grown in other tropical places like India. The biological activities of this plant are widely reported and it has been reported to possess anti-viral, anti-bacterial, anti-fungal and anti-inflammatory activities [4-6]. A study also reports the ability of extracts of *A. occidentale* to provide protection against streptozotocin-induced diabetes in rats [7]. The shoots of this plant extract inhibited copper-induced LDL oxidation [8]. The leaves of *A. occidentale* were reported to provide vasorelaxation effect when studied using isolated rat aorta [9]. The antioxidant activities and phenolic content of this plant have been reported mainly in the nuts and stem barks [10]. Recent studies have reported the antioxidant activities some extracts of the leaves of this plant [11]. But studies on the antioxidant activities of aqueous, ethanol, and petroleum ether (60-80^{0}C) extracts of the leaves of *A.occidentale* are not explored. In view of the limited data on the antioxidant activities of the leaves of *A. occidentale*, the present study was an attempt to determine the scavenging effects of the plant extracts on the free radical DPPH and NO-inhibition. The plant polyphenols may act as potent antioxidants and can prove beneficial for maintenance of optimal health, further assisting in providing evidence for the claimed medicinal properties of this plant.

MATERIALS AND METHODS

Plant Material

Plant samples were collected from Tungareshwar forests of Vasai Taluka, Dist. Thane in the state of Maharashtra, India. The plant specimen was authenticated at the Botanical Survey of India, Pune; (M.S). A herbarium of the plant specimen (specimen voucher number no. YOGA1/No.BSI/WC/Tech/2008/69) was submitted at the Botany Department of BSI, Pune.

Chemicals

All reagents and chemicals used in the experiments were of analytical grade and obtained from Sigma Aldrich (USA). Solvents used for extraction of plants were of analytical grade. Ascorbic acid used as a standard, DPPH (1,1-diphenyl-2-picrylhydrazyl), Sodium

nitroprusside, Sulphanilic acid and α-naphthyl-ethylene diamine were purchased from Sigma Aldrich (USA).

Preparation and extraction of plant extracts

Fully matured leaves of *A.occidentale* were collected, washed, and shade dried. The dried samples were ground to coarse powder form and extracted by Soxhlet extractor, below 50^0C with ethanol, and pet.ether (80-60^0C), for 18h with a mass to volume ratio of 1:6 (g/mL), except for aqueous extract which was prepared by refluxing for same duration and mass to volume ratio as the ethanolic and Pet.ether extract. The extracts were evaporated to dryness on the rotary evaporator, except for aqueous extract which was freeze dried.

Phytochemical investigation

The extracts were subjected to qualitative chemical tests [12,13] ethanol and aqueous extracts revealed the presence of tannins and phenolics, saponins, flavonoids, alkaloids, steroids and sugars. Pet.ether extract showed the presence of carbohydrates and alkaloids.

Determination of Total Polyphenol Content in various extracts

The total phenolic content was determined according to the Folin-Ciocalteu method [14]. The reaction mixture was composed of 0.1 mL of extract, 7.9 mL of distilled water, 0.2 mL of the Folin-Ciocalteu's reagent, and 1.5 mL of 20% sodium carbonate. The resultant solution was mixed and allowed to stand for 2 hours. The absorbance was measured at 765 nm in a Shimadzu UV- Spectrophotometer. The total phenolic content was determined as gallic acid equivalents (GAE)/mg of extract.

Free Radical Scavenging Activity

$DPPH^{\cdot}$ radical scavenging assay

Radical scavenging activities by antioxidants in the plant extracts were evaluated using $DPPH^{\cdot}$ (1,1-diphenyl-2-picrylhydrazyl) radicals [15]. Varying volumes of 0.2mg/mL the plant extracts were added to 200 μL of (0.36 mg/mL concentration) DPPH solution in methanol. A series of concentrations ranging from 2 to 15 μg of dried extracts were tested. The mixtures were shaken vigorously and incubated in the dark for 30 min after which the reduction of $DPPH^{\cdot}$ absorption was measured at 517 nm. Percent inhibition by sample treatment was determined by comparison with the methanol-treated control group. The IC_{50} values denote the concentration of each sample required to give 50% of the optical density shown by the control, using a non-linear regression analysis. All test analysis were run in triplicate and average values were reported. Ascorbic acid was used as positive control.

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Nitric oxide scavenging activity

The nitric oxide scavenging activity was conducted based on the Greiss Assay method [16]. 2.0 mL of 10 mM sodium nitroprusside and 5.0 mL of phosphate buffer were mixed with 0.5 mL of different concentrations of the plant extracts and incubated at 25^0C for 150 min. The sample was run as above but the blank was replaced with the same amount of water. After the incubation period, 2mL of the above incubated solution was added to 2 mL of Greiss reagent and incubated at room temperature for a period of 30 mins.

The absorbance of the chromophore formed was read at 540 nm. Ascorbic acid was used as positive control and results were expressed as percentage inhibition of nitric oxide. All determinations were performed in triplicates.

RESULTS

Nowadays, much research has been focused on reactive oxygen species and free radicals, which play a crucial role in the genesis of various diseases like inflammation, neurodegenerative complications, liver cirrhosis and cataract [17]. Herbal drugs possessing radical scavenging activity are gaining considerable importance in the treatment of such diseases. Phenolics and flavonoids are the major phytoconstituents responsible for the radical scavenging activity.

The phytochemical tests of ethanol and aqueous extracts revealed the presence of tannins and phenolics, saponins, flavonoids, alkaloids, steroids and sugars. Pet.ether extract showed the presence of carbohydrates and alkaloids.

Determination of Total Polyphenol Content

The total polyphenol content in the extracts were determined by Folin-Ciocalteu method. The total polyphenol content in the ethanol, and aqueous extracts were found to be 40.26 and 37.41 GAE/mg of extract respectively. The ethanol extract was found to contain the higher amount of polyphenol content as compared to aqueous extract.

Table 1: Total phenolic content of extracts of leaves of *A.occidentale*

Sr.No	Extract	Total phenolic content (GAE/mg of extract)
1	Aqueous	37.41
3	Ethanol	40.26

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Table 2: Antiradical activity of extracts of leaves of *A.occidentale* observed with DPPH Assay

Sample	Conc.(μg/mL)	% Inhibition ± SD (n=3)	IC_{50} value (μg)
Aqueous Extract	4	5.31 ± 0.08	
	6	16.20 ± 0.09	
	8	26.50 ± 0.09	12.76 μg
	10	35.82 ± 0.10	(r^2 =0.999)
	12	46.54 ±0.09	
	14	56.06 ± 0.09	
	16	64.36 ±0.08	
	18	67.28 ± 0.09	
	20	71.80 ± 0.08	
Ethanolic Extract	4	19.0 ± 0.01	
	6	33.10 ± 0.01	
	8	42.22 ± 0.02	9.41μg
	10	52.70 ± 0. 01	(r^2 =0.997)
	12	63.66 ± 0.02	
	14	73.83 ± 0.03	
	16	81.96 ± 0.01	
	18	88.24 ± 0.02	
	20	89.80 ± 0.01	
Pet. Ether Extract	4-20	na	-
Ascorbic acid (positive control)	4	40.30 ± 0.02	
	6	51.41 ± 0.01	
	8	67.70 ± 0.01	5.30μg
	10	87.79 ± 0.02	(r^2 =0.991)
	12	91.50 ± 0.01	
	14	95.70 ± 0.03	
	16	95.80 ± 0.02	
	18	95.64 ± 0.01	
	20	95.88 ± 0.02	

n = 3, Values are means of 3 replicate determinations. na = no activity

r^2 = regression coefficient determined for 5 concentrations.

DPPH· radical scavenging assay

The radical scavenging activities of the extracts of *A. occidentale* were estimated by comparing the percentage inhibition of formation of DPPH· radicals by the extracts and those of Ascorbic acid. **Fig. 1** depicts a steady increase in the percentage inhibitions of the absorbance of the DPPH radicals by the extracts up to a concentration of 16.0 μg/mL, and 18.0 μg/mL for aqueous and ethanolic extracts respectively, after which there was a leveling off with much slower increase in inhibition. This pattern of DPPH inhibition is commonly observed with plant extracts [18-20]. An exception was observed in the Pet.ether (60-80^{0}C) extract which did not show a considerable linear increase in DPPH scavenging activity with increasing concentration, and thus its radical scavenging activity results are not illustrated. Overall, the Aqueous and Ethanol extracts of *A. occidentale* were able to inhibit the formation of DPPH· radicals. The aqueous extract and ethanol extracts of *A.occidentale* had IC_{50} values of 12.76 μg and 9.41μg

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respectively which is inversely related to its antioxidant ability. The IC_{50} value of Ascorbic acid (standard) was found to be 5.30μg. Based upon the IC_{50} values of the extracts it can be concluded that, ethanol extract is more potent as an antioxidant than aqueous extract. The $DPPH^{\cdot}$ radical scavenging capacity of the extracts of *A. occidentale* are at twice the concentration, observed for Ascorbic acid **Table 2**. This suggests that *A. occidentale* can potentially exert its radical scavenging effects at a much lower concentration.

Table 3: *In vitro* Nitric oxide Scavenging activity of extracts of leaves of *A.occidentale*

Sample	Conc.(μg/mL)	% Inhibition ± SD (n=3)	IC_{50} value (μg)
Aqueous Extract	800 900 1000 1100 1200	28.51 ± 0.006 38.30 ± 0.005 46.90 ± 0.002 63.80 ± 0.004 71.14 ± 0.007	1002.43 (r^2 =0.992)
Ethanolic Extract	100 300 600 900 1200	28.51 ± 0.002 38.30 ± 0.002 46.90 ± 0.003 63.80 ± 0.009 71.14 ± 0.004	658.30 (r^2 =0.994)
Pet. Ether Extract	100-1200	na	-
Ascorbic acid (positive control)	100.00 300.00 600.00 900.00 1200.00	7.8± 0.005 29.9 ± 0.006 48. 0 ± 0.005 65.60 ± 0.02 85.7 ± 0.004	325.17 (r^2 =0.992)

n = 3, Values are means of 3 replicate determinations. na = no activity. r^2 = regression coefficient determined for 5 concentrations.

Nitric oxide scavenging activity

Sodium nitro-prusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Overall, the ethanol extract of *A. occidentale* showed higher nitric oxide scavenging ability compared to the aqueous extract **Fig. 2**. The IC_{50} values of ethanol and aqueous extracts were found to be 658.3 μg and 1002.3 μg of dry extract respectively. However pet.ether extract did not show any considerable inhibition of nitric oxide. The result of the nitric oxide scavenging potential of the extracts is shown in **Table 3**.

DISCUSSION

This study showed that among the aqueous, ethanol, and pet.ether (60-80^{0}C) extracts of *A. occidentale*, the Ethanolic extract possesses significant antioxidant activity and the potency of the extracts is in the order of ethanol > aqueous. Pet.ether extract did not show any

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Fig.No 1: DPPH Radical Scavenging capacity of extracts of Leaves of *A. occidentale*

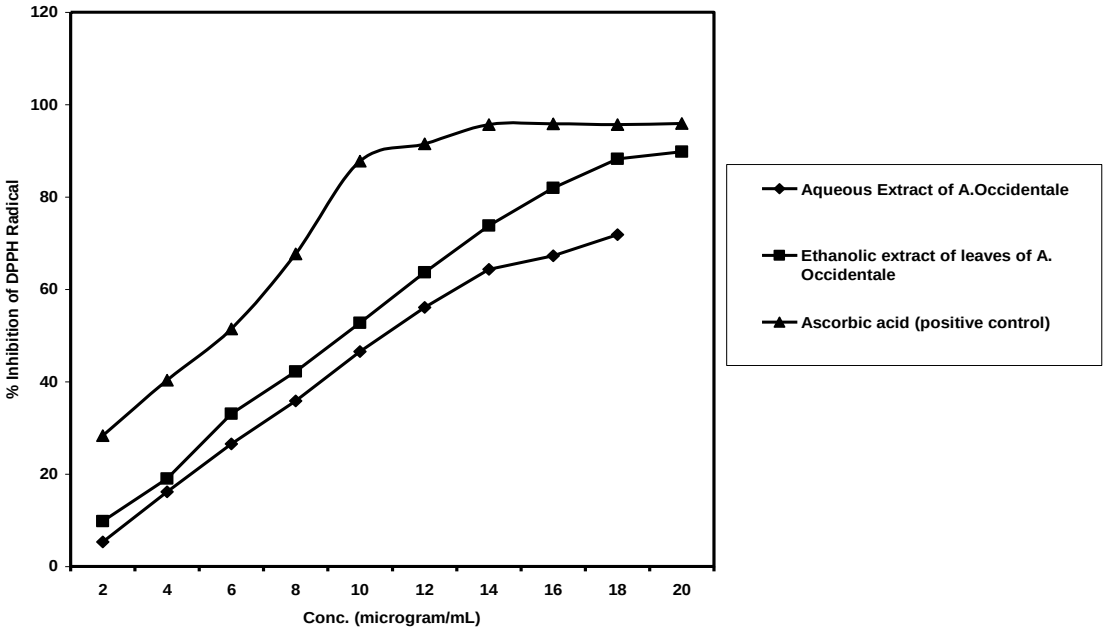
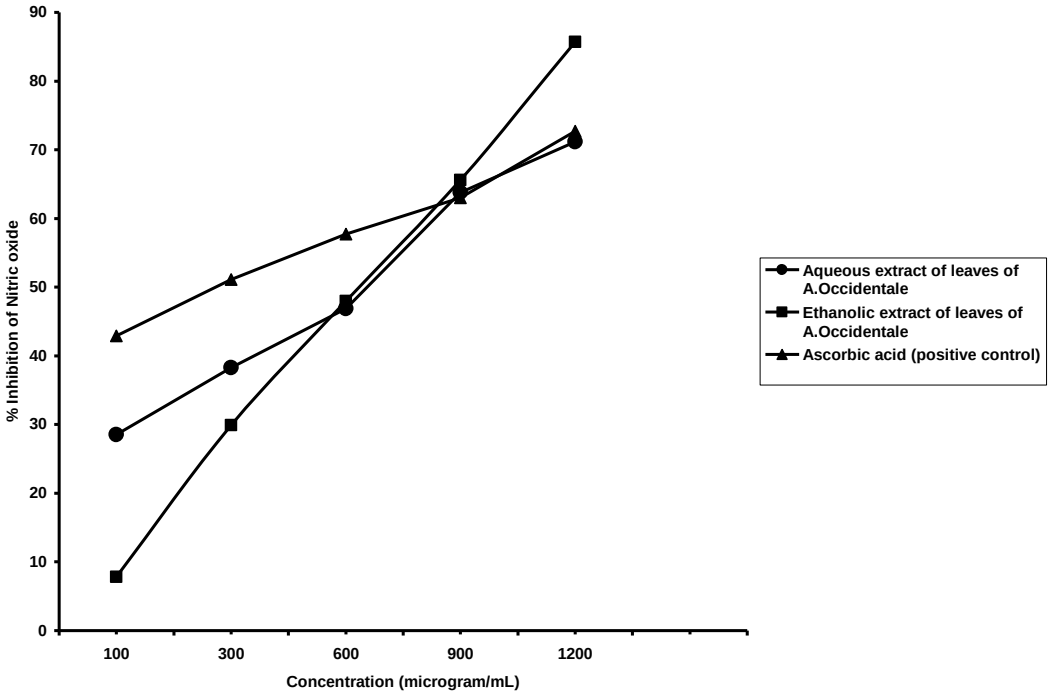


Fig.No 2: Nitric oxide Scavenging capacity of extracts of Leaves of *A. occidentale*



antioxidant activity in the DPPH and nitric oxide radical scavenging assay. Overall, the ethanol extract of *A. occidentale* is the most potent in scavenging the DPPH and nitric oxide. The presence of high levels of phenolic compounds in the ethanol extract may have partly contributed to the observed antioxidant activities. This study provided evidence on the potential health benefits of *A. occidentale*. Characterization of active constituents responsible for this activity and *In -vivo* studies are further required to understand the mechanism of action of this plant as an antioxidant.

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