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Antioxidant Activities and GCMS Analysis of *Anacardium occidentale* L. Fruits

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ABSTRACT

Anacardium occidentale belongs to the family Anacardiaceae is a tropical evergreen tree with spreading branches which are supported by aerial roots that go down into the soil to form the 'props'. The milky latex is applied externally for treating pains in rheumatism and in lumbago. The infusion of the bark is used against dysentery, diarrhoea and diabetes. The study aims to evaluate the antioxidant activities and GC-MS analysis of ethanol and aqueous fruit extracts of *Anacardium occidentale.* The antioxidant assays such as DPPH˙ radical, Superoxide (O_2-) radical, $ABTS^{\bullet+}$ radical cation scavenging activities, phoshomolybdenum reduction and Fe^{3+} reduction activities were carried out for ethanol and aqueous fruit extracts. The maximum DPPH˙ radical scavenging activity for ethanol extract was 93.33±0.23 at 120 µg/mL concentration and the IC_{50} was 38.29 µg/mL concentration. The maximum superoxide (O_2-) radical scavenging activity was 75±0.25 at 120 µg/mL concentration and the IC_{50} was 38.52 µg/mL concentration for ethanol extract. The maximum $ABTS^{\bullet+}$ radical cation scavenging activity for ethanol extract was 82.57±0.37 at 30 µg/mL concentration and the IC_{50} was 11.13 µg/mL concentration. The maximum phosphomolybdenum reduction was 92.94±0.19 at 120 µg/mL concentration and the RC_{50} was 17.75 µg/mL concentration for ethanol extract. The maximum Fe^{3+} reduction for ethanol extract was 75.99±0.31 at 120 µg/mL concentration and the RC_{50} was 40.48 µg/mL concentration. GC-MS analysis showed different ester derivative compounds present in the ethanol fruit extract of *Anacardium occidentale* exhibiting antioxidant, anti-inflammatory, antimicrobial activities.

Keywords: *Anacardium occidentale*, DPPH˙ radical, Superoxide (O_2-) radical, $ABTS^{\bullet+}$ radical cation GC-MS analysis.

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INTRODUCTION

Anacardium occidentale L. (cashew) of the family Anacardiaceae is native to Brazil. It is a small-sized tree with a dome-shaped crown. The bark is brown or grey, and smooth to rough with longitudinal fissures[1,2]. Leaves are leathery and obovate with a rounded apex. Borne on terminal clusters, leaves are pliable, lustrous and reddish when young, and dark green when mature with prominent yellow veins. Occurring as terminal panicles, flowers are whitish turning pinkish-red. Fruits are a kidney-shaped nut (Figure 1) attached to the distal end of an enlarged pear-shaped receptacle called the cashew apple.

The true fruit of the cashew tree is a kidney or boxing-glove shaped drupe that grows at the end of the cashew apple. The drupe develops first on the tree, and then the pedicel expands into the cashew apple. Within the true fruit is a single seed, the cashew nut. The fruit is thick, with oval seeds, 2-3 cm in length. The seeds are reddish brown-skinned with two large cotyledons and a small embryo. The fruit bark juice and nut oil are used as home remedies for calluses, corn, warts, cancerous ulcers and elephantiasis[3,4]. According to Ayurveda, fruit can be used in the treatment of anthelmintic, aphrodisiac, ascites, fever, dysentery, piles, Leucoderma, etc. The true fruit of cashew releases a liquid rich in phenolic compounds, known as liquid from chestnut shell- cashew nut shell liquid (CNSL). Both yellow and red fruits of *A. occidentale* possess ferulic acid, caffeic acid, sinapic acid, gallic acid, and ellagic myritine[5,6,7].

Molecules with an unpaired electron are said to be free radicals. These molecules are highly reactive species due to the presence of a free electron. They are highly important in natural mechanism involved in neurotransmission, vascular tone control and cytotoxic profile. Nowadays the research is focussed on food derived products in order to derive phyto-

molecules that could be turned as an active-drug with effective therapeutic properties[8].

Taxonomic classification of *Anacardium occidentale*

Kingdom: Plantae

Division: Angiosperms

Class: Eudicots

Sub class: Rosids

Order: Sapindales

Family: Anacardiaceae

Genus: *Anacardium*

Species: *A. occidentale*

Figure 1: *Anacardium occidentale* fruits

MATERIALS AND METHODS

Collection of fruits and preparation of the extract

A. occidentale fruits were collected at Porur, Chennai, India. The fruits were washed with tap water, cut into small pieces and soaked in ethanol for 72 h. The aqueous extract was prepared by hot decoction method. The ethanol and aqueous supernatant were filtered separately by sterile filter paper and condensed by using rotary evaporator 50°C, which yields gummy extract[9,10]. The fruit extracts of *A. occidentale* was subjected to *in vitro* antioxidant activities and quantitative phytochemical analysis.

In vitro antioxidant activities

DPPH˙ radical scavenging activity

The antioxidant activity of ethanol and aqueous fruit extracts of *A.occidentale* was measured on the basis of stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) radical scavenging activity [11]. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (20-120 µg/mL) of ethanol and aqueous fruit extracts. The mixture was then allowed to stand for 30 min incubation in dark. Ascorbic acid was used as the reference standard. One mL methanol and 1 mL DPPH solution was used as the control. The decrease in absorbance was measured at 517 nm. The percentage of inhibition was calculated as:

$$\% \text{ of DPPH}^{\cdot} \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100\right]$$

Superoxide (O_2-) radical scavenging activity

The superoxide (O_2-) radical scavenging activity of ethanol and aqueous fruit extracts of *A.occidentale* was measured on the basis of superoxide radical inhibition[12]. Various concentrations (20-120 µg/mL) of ethanol and aqueous fruit extracts were mixed with 50 mM phosphate buffer (pH-7.6). The mixtures were combined with 200 µL of 1.5 mM Riboflavin, 200 µL of 12 mM EDTA followed by 100 µL of 50 mM NBT. The reaction was started by illuminating the test tubes in UV-lamp for 15 min. The superoxide (O_2-) radical reduces NBT to a blue colored formazon can be measured at 590 nm. Ascorbic acid was used as the reference standard. The percentage of superoxide (O_2-) radical inhibition can be calculated as:

$$\% \text{ of superoxide } (O_2\text{-}) \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}} *100\right]$$

$ABTS^{\bullet+}$ radical cation scavenging activity

The antioxidant capacity was estimated in terms of the $ABTS^{\bullet+}$ radical cation scavenging activity following the procedure[13]. $ABTS^{\bullet+}$ was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12-16 h before use. The $ABTS^{\bullet+}$ solution (stable for 2 days) was diluted with distilled water to reach an absorbance of 0.70±0.02 at 734 nm. Various concentrations (5-30 µg/mL) of ethanol and aqueous fruit extracts was mixed with 500 µL of diluted $ABTS^{\bullet+}$ solution and the absorbance was measured at 734 nm after 10 min. Ascorbic acid was used as the reference standard. The $ABTS^{\bullet+}$ radical-scavenging activity was expressed as

$$\% \text{ of } ABTS^{\bullet+} \text{ radical cation inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}} *100\right]$$

Phosphomolybdenum reduction activity

The antioxidant capacity of ethanol and aqueous fruit extracts of *A. occidentale* was assessed by the method[14]. The fruit extracts with different concentrations ranging from 20-120 µg/mL was combined with 1 mL of reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 95°C for 90 min. The absorbance of the coloured complex was measured at 695 nm. Ascorbic acid was used as standard reference. The percentage of reduction was calculated as:

$$\% \text{ of } Mo^{6+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} *100\right]$$

Ferric (Fe^{3+}) reducing power activity

The reducing power of ethanol and aqueous fruit extracts of *A. occidentale* was determined by slightly modified method[15]. One mL of ethanol and aqueous fruit extracts of different concentrations (20-120 µg/mL) was mixed with 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%, w/v) solution and 1 mL of 0.2 M phosphate buffer (pH 6.6) solution. The mixture was then incubated at 50°C for 30 min in water bath. Five hundred µL of trichloroacetic acid (10% w/v) was added to each mixture. Then 100 µL of freshly prepared $FeCl_3$ (0.1%, w/v) solution was added and the absorbance was measured at 700 nm. Ascorbic acid was used as the standard reference.

$$\% \text{ of } Fe^{3+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} *100\right]$$

Estimation of total phenolic content

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds with slight modifications[16]. One hundred µL of ethanol fruit extract (1 mg/mL) of *A. occidentale* was mixed with 900 µL of ethanol and 1 mL of Folin Ciocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of 20% (w/v) Na_2CO_3 solution was added. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference compound.

Estimation of total flavonoids content

The total flavonoid content of ethanol fruit extract of *A. occidentale* was determined using aluminium chloride reagent method with slight modifications[17]. Five hundred µL of ethanol fruit extract (1 mg/mL) was mixed with 500 µL of ethanol and 0.5 mL of 5% (w/v) sodium nitrite solution. Then, 0.5 mL 10% (w/v) aluminium chloride solution was added and incubated for 5 min at room temperature followed by 100 µL of 1 M NaOH solution was added. Absorbance was measured at 510 nm using spectrophotometer. The result was expressed as (µg/mg of extract) quercetin equivalent.

Gas chromatography–Mass Spectrometry (GC–MS) analysis

For GC-MS analysis, the ethanol fruit extract were injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units[18].

RESULTS AND DISCUSSION

Invitro antioxidant activities

DPPH˙ radical scavenging activity

The ability of the ethanol extract of *A.occidentale* to scavenge DPPH˙ (1,1-diphenyl-2-picrylhydrazyl) was higher than aqueous extract of *A.occidentale* comparatively. The maximum DPPH˙ radical scavenging activity for the ethanol extract was found to be 93.33±0.23% and for the aqueous extract as 85.38±0.47% at 120 µg/mL concentration. Ethanol extract of *A.occidentale* demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2- picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract. The IC_{50} value of the ethanol extract of *A.occidentale* was found to be 38.29 µg/mL concentration (Table 1 and Graph 1) and was compared with standard (Ascorbic acid, IC_{50} = 11.92 µg/mL concentration).

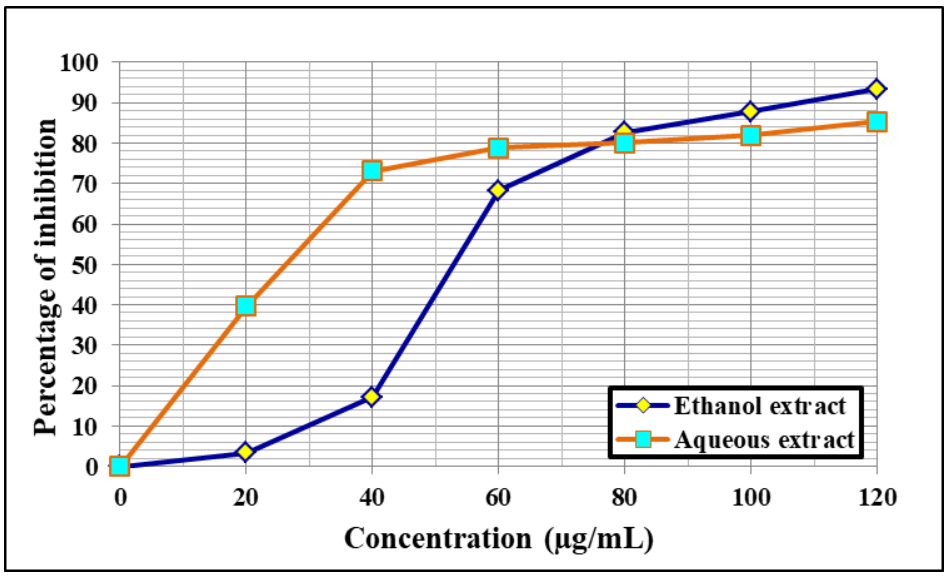
Superoxide (O_2-) radical scavenging activity

Superoxide anion is also very harmful to cellular components and their effects can be magnified because it produces other kinds of free radicals and oxidizing agents[19]. Flavonoids are effective antioxidants, mainly because they scavenge superoxide anions. Superoxide anions derived from dissolved oxygen by the riboflavin-light-NBT system will reduce NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT^{2+}) to blue formazan, which is measured at 590 nm in UV-Vis spectrophotometer. Antioxidants are able to inhibit the blue NBT formation and the decrease of absorbance with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The maximum superoxide ($O_2^{\cdot-}$) radical scavenging activity for the ethanol extract was found to be 75.00±0.25% and for the aqueous extract as 57.97±0.19% at 120 µg/mL concentration (Table 1 and Graph 2) and the IC_{50} value of the ethanol extract of *A.occidentale* was found to be 38.52 µg/mL concentration. It was compared with the standard of ascorbic acid (IC_{50} = 10.67 µg/mL concentration).

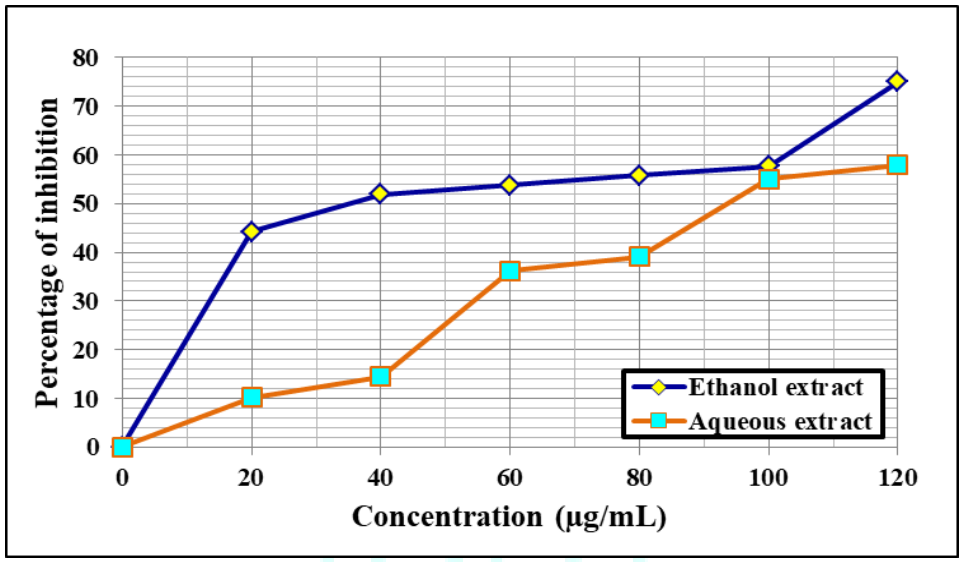
Table 1: DPPH˙ radical and superoxide ($O_2^{\cdot-}$) radical scavenging activity of ethanol and aqueous fruit extracts of *A. occidentale*

S. No	Concentration (µg/mL)	% of inhibition*			
		Ethanol Extract		Aqueous Extract	
		DPPH˙ radical	Superoxide ($O_2^{\cdot-}$ radical)	DPPH˙ radical	Superoxide ($O_2^{\cdot-}$ radical)
1	20	3.33±0.23	44.23±0.19	39.61±0.27	10.14±0.20
2	40	17.22±0.20	51.92±0.45	73.07±0.11	14.49±0.11
3	60	78.33±0.48	53.84±0.32	78.84±0.41	36.23 ±0.33
4	80	82.77±0.19	55.76±0.17	80.00±0.20	39.13±0.46
5	100	87.77±0.14	57.69±0.39	81.92±0.23	55.07±0.17
6	120	93.33±0.23	75.00±0.25	85.38±0.47	57.97±0.19

(*Average value of 3 replicates)



Graph 1: DPPH˙ radical scavenging activity of ethanol and aqueous fruit extracts of *A. occidentale*



Graph 2: Superoxide ($O_2^{\cdot-}$) radical scavenging activity of ethanol and aqueous fruit extracts of *A. occidentale*

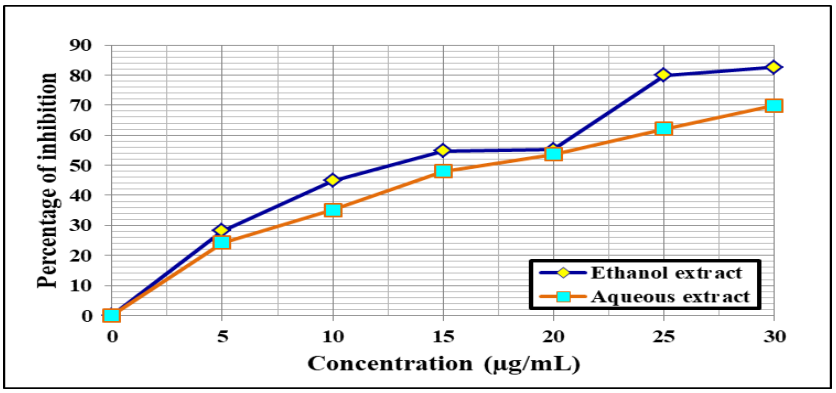
ABTS$^{\bullet+}$ radical cation scavenging activity

ABTS$^{\bullet+}$ is a blue-green chromophore produced by the reaction between ABTS and potassium persulfate. The blue green chromophore radical cation gets reduced while reacting an antioxidant in the ethanol and aqueous fruit extracts of *A.occidentale* and the remaining radical cation concentration was quantified with the loss of colour[20]. The antioxidant reduces ABTS$^{\bullet+}$ to ABTS and decolorize the blue-green chromophore. The maximum ABTS$^{\bullet+}$ radical cation scavenging activity was found to be 82.57±0.37% for the ethanol extract and for the aqueous extract as 69.93±0.12% at 30 µg/mL concentration (Table 2 and Graph 3) with the IC_{50} of 11.13 µg/mL concentration for the ethanol extract of *A.occidentale* and was compared with standard ascorbic acid (IC_{50} = 4.82 µg/mL concentration).

Table 2: ABTS$^{\bullet+}$ radical cation scavenging activity of ethanol and aqueous fruit extracts of *Anacardium occidentale*

S. No	Concentration (µg/mL)	% of inhibition*	
		ABTS$^{\bullet+}$ radical cation	
		Ethanol Extract	Aqueous Extract
1	5	28.17±0.47	24.29±0.23
2	10	44.89±0.24	35.18±0.41
3	15	54.80±0.36	48.02±0.17
4	20	55.11±0.15	53.67±0.38
5	25	79.92±0.29	62.14±0.42
6	30	82.57±0.37	69.93±0.12

(*Average value of 3 replicates)



Graph 3: ABTS$^{\bullet+}$ radical cation scavenging activity of ethanol and aqueous fruit extracts of *Anacardium occidentale*

Phosphomolybdenum reduction activity

Metal-Catalyzed Oxidation (MCO) systems catalyze the reduction reaction, which alters the nature of proteins at the metal-binding site and cause DNA and protein damage. The total antioxidant activity of the ethanol and aqueous fruit extracts of *A.occidentale* was measured by phopho molybdenum reduction method which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm[21]. The maximum phosphor molybdenum reduction was 92.94±0.19% for the ethanol extract and for the aqueous extract as 97.14±0.46% at 120 µg/mL concentration (Table 3 and Graph 4) with the RC_{50} of 17.75 µg/mL concentration for the ethanol extract of *A.occidentale*. It was compared with the standard ascorbic acid (RC_{50} = 7.91 µg/mL).

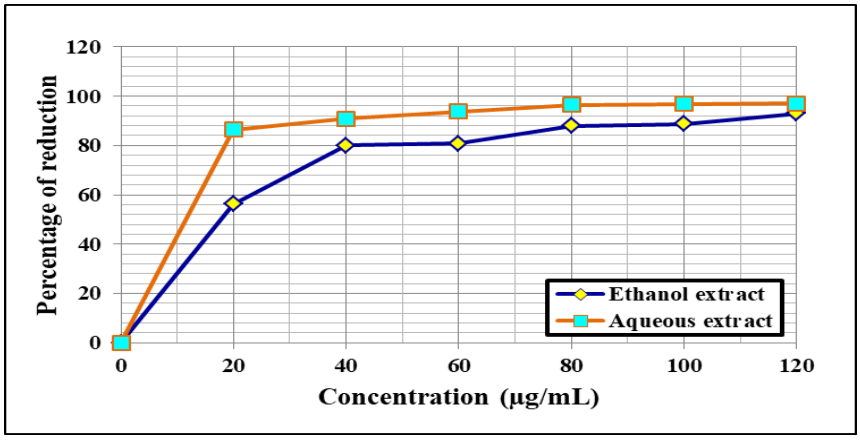
Ferric (Fe^{3+}) reducing power activity

The reducing power assay was carried out by the reduction of Fe^{3+} to Fe^{2+} by the ethanol and aqueous fruit extracts of *A. occidentale* and the subsequent formation of ferro-ferric complex. Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action[22]. The reducing ability of a compound generally depends on the presence of reductones (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom or by neutralizing the free radicals by donating an electron and become lone pair of electrons instead of odd electron. The reduction ability increases with increase in concentration of the extracts. The maximum Fe^{3+} reduction was 75.99 ± 0.31% for the ethanol extract and 85.18 ± 0.20% for the aqueous extract at 120 µg/mL concentration (Table 3 and Graph 5) with the RC_{50} of 40.48 µg/mL concentration for the ethanol extract of *A.occidentale* and was compared with the standard ascorbic acid (RC_{50} = 9.39 µg/mL).

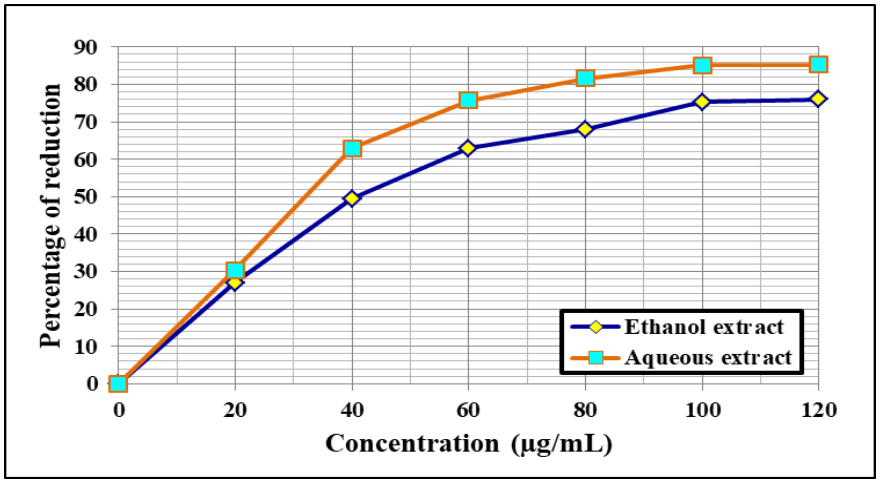
Table 3: Phosphomolybdenum reduction and Fe^{3+} reducing power activity of ethanol and aqueous fruit extracts of *A. occidentale*

S. No	Concentration (µg/mL)	% of reduction*			
		Ethanol Extract		Aqueous Extract	
		Mo^{6+} reduction	Fe^{3+} reduction	Mo^{6+} reduction	Fe^{3+} reduction
1	20	56.31 ± 0.35	26.94 ± 0.18	86.48 ± 0.15	30.39 ± 0.12
2	40	80.00 ± 0.20	49.40 ± 0.45	90.78 ± 0.27	62.90 ± 0.41
3	60	80.76 ± 0.42	62.92 ± 0.40	93.77 ± 0.33	75.76 ± 0.38
4	80	87.93 ± 0.15	67.92 ± 0.26	96.44 ± 0.19	81.57 ± 0.11
5	100	88.66 ± 0.26	75.28 ± 0.39	96.88 ± 0.26	85.06 ± 0.18
6	120	92.94 ± 0.19	75.99 ± 0.31	97.14 ± 0.46	85.18 ± 0.20

(*Average value of 3 replicates)



Graph 4: Phosphomolybdenum reduction activity of ethanol and aqueous fruit extracts of *A. occidentale*



Graph 5: Fe^{3+} reducing power activity of ethanol and aqueous fruit extracts of *A. occidentale*

Total Phenols and Flavonoids content

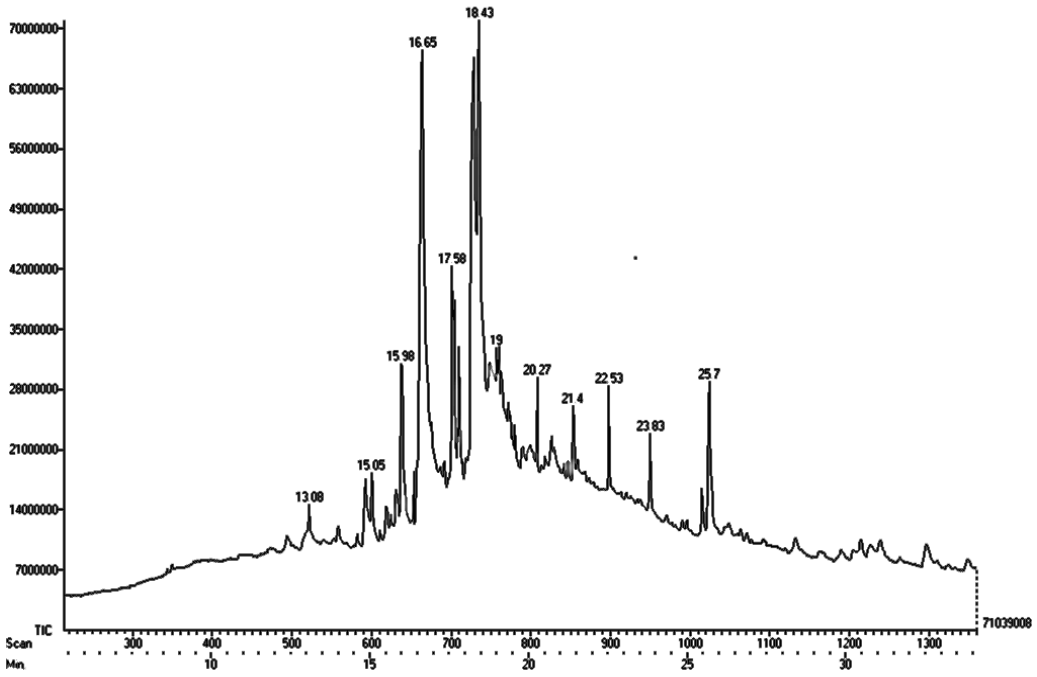
Based on the *invitro* antioxidant activities, ethanol fruit extract of *A. occidentale* was selected for further analysis. Flavonoids and phenolic compounds are known to play major roles in the antioxidant and prooxidant capacities exhibited by plant extracts. The antioxidant effect conferred by these compounds are due to the phenolic hydroxyl groups attached to their respective ring structures that can act as reducing agents, hydrogen donors, singlet oxygen quenchers, super oxide radical scavengers, and as metal chelators. They are also said to reduce α–tocopherol radicals or tocopheroxyls, activate antioxidant enzymes, mitigate nitrosative stress, and inhibit oxidases[23]. After proton donation, these compounds are oxidized to resonance-stabilized radicals that can further act as prooxidants at high concentrations, high pH, and in the presence of metal ions. Flavonoids and phenolic acids present in food such as quercetin, myricetin, caffeic acid, gallic acid, chlorogenic acid, coumaric acid, ferulic acid, and ellagic acid have been proven to exhibit dual *in vitro* behavior[24]. The total phenolic and flavonoid content of ethanol fruit extract of *Anacardium occidentale* were 421.54±0.29 µg/mg GAE and 15.54±0.18 µg/mg QE respectively.

Gas chromatography–Mass Spectrometry (GC–MS) analysis

GC-MS analysis was carried out for the ethanol extract of *A. occidentale* and the eluted compounds were showed in Table 4. Two compounds namely 14,17- octadecadienoic acid, methyl ester exhibits skin scaling and wound healing property. Heptadecanoic acid,16 methyl,methyl ester was reported to exhibit antioxidant, anti–inflammatory, anti-fibrotic and vasodilator property (Table 5 and Graph 6) which were confirmed by GC-MS analysis. These potential compounds could be one of the reasons for the antioxidant property of the ethanol fruit extract of *Anacardium occidentale*.

Table 4: Active compounds identified in ethanol fruit extract of *Anacardium occidentale* by GC-MS analysis

S. No	RT	Compound Name	Mol. Formula	Mol.weight (g/mol)	Compound Structure
1	15		$C_{13}H_{26}O_2$	214.34	Undecanoic acid,10-methyl,methyl ester
2	14.17		$C_{19}H_{34}O_2$	294.47	14,17-octadecadienoic acid, methyl ester
3	19.07		$C_{19}H_{38}O_2$	298.50	Heptadecanoic acid,16 methyl, methyl ester
4	20.55		$C_{19}H_{36}O_3$	312.49	Oxiraneoctanoic acid,3 octyl,methyl ester
5	16.13		$C_{14}H_{24}O_4$	256.33	5-Decenedioic acid,5,6-dimethyl,dimethyl ester
6	17.08		$C_{17}H_{34}O_2$	270.45	Hexadecanoic acid, methyl ester
7	18.13		$C_{18}H_{36}O_2$	284.47	Hexadecanoic acid,14-methyl,methyl ester



Graph 6: GCMS Chromatogram of ethanol fruit extract of *Anacardium occidentale*

Table 5: Pharmacological activities of ethanol fruit extract of *Anacardium occidentale*

S.No	Compound Name	Pharmacological activity[25,26]
1	14,17-octadecadienoic acid, methyl ester	Prevent hair loss Skin scaling Wound healing
2	Heptadecanoic acid,16 methyl, methyl ester	Antioxidant Anti-inflammatory Anti-fibrotic Vasodilator
3	Hexadecanoic acid, methyl ester	Antioxidant Antifungal
4	Hexadecanoic acid,14-methyl, methyl ester	Antioxidant Anti-inflammatory Hypocholesterolemic Nematicide Pesticide anti-androgenic

CONCLUSION

From the present investigation, the *Anacardium occidentale* fruits possessed significant antioxidant activities for different extracts, thereby proving that the fruits contain rich-antioxidant molecules on natural basis. The *Anacardium occidentale* fruits contained higher amount of phenolic compounds and moderate amount of flavonoid content. Natural products in the pharmaceutical industry have reduced, owing to issues such as the lack of compatibility of traditional natural product extract libraries with high-throughput screening.Further, the individual active compounds can be isolated by chromatographic techniques and the fractions shall be evaluated separately for FTIR, NMR to identify the compound functional group, nature and structure for converting as a new active drug.

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REFERENCES

1. Eric Wei Chiang Chan, Shigeyuki Baba, Hung Tuck Chan, Mami Kainuma, Tomomi Inoue, Siu Kuin Wong. Ulam herbs: A review on the medicinal properties of *Anacardium occidentale* and *Barringtonia racemosa*. Journal of Applied Pharmaceutical Science, 2017; 7(02):241-247.
2. Lim TK. *Anacardium occidentale*. In: Edible Medicinal and Non-medicinal Plants, Vol. I, Fruits. Dordrecht, Heidelberg, London and New York: Springer Science and Business Media BV, 2012; 45-68.
3. Brijyog, Laliteshwar pratap singh, Anup maiti. Pharmacological Importance of *Anacardium occidentale*: A Review. Asian Journal of Pharmaceutical Education and Research, 2017; 6(1).
4. Patro C and Behera RN. Cashew helps to fix sand dunes in Orissa. Indian Farming, 1979; 28(12):31–32.
5. Aracelli de Sousa Leite, Md. Torequl Islam1, Antonio Luiz Gomes Júnior, João Marcelo de Castro e Sousa, Marcus Vinícius Oliveira Barros de Alencar, Márcia Fernanda Correia Jardim Paz, Hercília Maria Lins Rolim, Maria das Graças Freire de Medeiros, Ana Amélia de Carvalho Melo-Cavalcante and José Arimatéia Dantas Lopes. Pharmacological properties of cashew (*Anacardium occidentale*). African Journal of Biotechnology, 2016; 15(35):1855-1863.
6. Kumar P, Paramashivappa R, Vithayathil P, Rao PS, Rao AS. Process for isolation of cardanol from technical cashew

(*Anacardium occidentale* L.) nut shell liquid. J. Agric. Food Chem, 2012; 50:4705-4708.
7. Moo-Huchin VM, Moo-Huchin MI, Estrada-Leon RJ, Cuevas-Glory L, Estrada-Mota IA, Ortiz-Vazquez E, Betancur-Ancona D, Sauri-Duch E. Antioxidant compounds, antioxidant activity and phenolic content in peel from three tropical fruits from Yucatan, Mexico. Food Chem, 2015; 166:17-22.
8. Abheri Das Sarma, Anisur Rahaman Mallick and Ghosh AK. Free Radicals and Their Role in Different Clinical Conditions: An Overview. International Journal of Pharma Sciences and Research, 2010; 1(3):185- 192.
9. Harborne JB, Phytochemical Methods, A guide to Modern Techniques of Plant analysis, second ed. Chapman and Hall, London, 1998; 54-84.
10. Raaman N. Phytochemical techniques. New India Publishing Agency, New Delhi, 2006; 306.
11. Liu, X., Dong, M., Chen, X., Jiang, M., Lv, X. and Yan, G. Antioxidant activity and phenolics of endophytic *Xylaria* sp. From *Ginkgo biloba*. Food Chemistry, 2007; 105:548-554.
12. Spanos GA and Wrosltad RE. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. Journal of Agricultural & Food Chemistry, 1990; 38:1565-1571.
13. Khalaf NA, Shakya AK, Al-othman A, El-agbar Z, Farah H. Antioxidant activity of some common plant. Turk J Biol, 2008; 32:51-5.
14. Lokesh Deb SK, Dubey, Avijeet Jain, Amit Kumar Jain, Pandian GS. Free radical scavenging activity of aqueous n- butanol fraction of *Prunus Persica* aqueous extract. Journal of Natural Remedies, 2009; 9(2):152-158.
15. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem, 2001; 73:239-44.
16. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry, 1999; 269:337-341.
17. Oyaizu M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr, 1986; 44:307315.
18. Harini V, Vijayalakshmi M, Sivaraj C, Arumugam P. Antioxidant and Anticancer Activities of methanol Extract of *Melochia corchorifolia* L. Int. J. of Sci. and Res, 2017; 6(1):1310-1316.
19. Wickens AP. Aging and the free radical theory, Respiratory Physiology. 2001; 128:379-391.
20. Miller DD. Mineral. In: Fennema, O.R. (Ed.), *Food Chemistry* 1996; Marcel Deckker, New York, 618-649.
21. Yıldırım A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts. J. Agric. Food Chem, 2001; 49:4083-4089.
22. Stadtman ER. Metal ion-catalyzed oxidation of proteins: Biochemical mechanism and biological consequences. Free Radical Biology and Medicine, 1990; 9:315-325.
23. D. Prochazkova I. Bousova, N. Wilhelmova. Antioxidant and prooxidant properties of flavonoids. *Fitoterapia* 2011; 82:513–523.
24. Eboh Abraham Sisein, Biochemistry of Free Radicals and Antioxidants. Sch. Acad. J. Biosci, 2014; 2(2):110-118.
25. Milena Cotoras, Herman Vivanco, Ricardo Melo, María Aguirre, Evelyn Silva and Leonora Mendoza. In Vitro and in Vivo Evaluation of the Antioxidant and Prooxidant Activity of Phenolic Compounds obtained from Grape (*Vitis vinifera*) Pomace. Molecules, 2014; 19, 21154-21167.
26. Muniyandi Anbukkarasi, Philip A Thomas, Mahalingam Sundararajan, Pitchairaj Geraldine. Gas Chromatography - Mass Spectrometry Analysis and In vitro Antioxidant Activity of the Ethanolic Extract of the Leaves of *Tabernaemontana divaricata.* Pharmacogn. J, 2016; 8(5):451-458.