Research Article

Detection of Phenolics and Appraisal of Antioxidant and Antimicrobial Properties of *Arenga wightii*

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ABSTRACT

In this study, we report the antioxidative potential of the methanolic extract of endemic palm *Arenga wightii* Griff. By various antioxidant assays including DPPH (2, 2-Diphenyl-1-picrylhydrazyl), ABTS (2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), nitric oxide, lipid peroxidation and reducing power activity. Fruits exhibited high reduction capability and powerful free radical scavenging ability compared to leaf extract in all the antioxidant assays except ABTS assay. Total phenolics and flavonoid content of plant extract was also determined by a colorimetric method and have significant amount of phenolics and flavonoids which are responsible for strong antioxidant and antibacterial activities. The antibiotic activity of both fruit and leaf extract was more pronounced against *Staphylococcus aureus*. RP-HPLC analysis revealed the presence of major phenolic compounds like gallic acid, ascorbic acid, chlorogenic acid and caffeine in the leaf extract. GC-MS analysis was done for identification of chemical compounds after silylation. Based on the results, we suggest that *A. wightii* possess high phenolics and other phytocompounds which exhibit effective antioxidant and antibacterial properties.

Keywords: ABTS, *A. wightii*, Antioxidant activity, DPPH, GC-MS, RP- HPLC.

INTRODUCTION

Reactive oxygen species such as the superoxide anion (O^-) hydrogen peroxide (H_2O_2) peroxyl radicals (ROO^-), reactive hydroxyl (OH^-) and nitric oxide (NO^-) radicals are continuously generated as a byproduct during electron transport chain and normal cell metabolism.[1] An imbalance between the generation of reactive oxygen and nitrogen species (ROS/RNS) is defined as oxidative stress which plays a vital role in different pathological conditions such as cancer, diabetes, aging and other degenerative diseases in humans.[2-4] Antioxidants are the only effective agents that have been reported to prevent oxidative damage caused by free radicals and have the ability to interfere with the oxidation process by reacting with free radicals and quenching singlet oxygen molecules.[5,6] On the other hand, synthetic antioxidants like butylated hydroxyanisole, butylated hydroxytoluene was found to exert carcinogenic potential. Plant derived drugs are gaining popularity as an alternative form of health care. So far, numerous researchers have revealed a great deal of drugs because of their better safety, efficacy and wide acceptance by the consumers.[7]

A. wightii is a palm belongs to the family Arecaceae (Palmae) found in the slopes of Western Ghats of Kerala in India.[8] Different parts of *A. wightii* were reported to possess various medicinal properties. Starch prepared from pith was administered orally for painful urination and leucorrhoea. Sap collected from inflorescence is used as a cooling anti diarrheal agent. Fresh toddy obtained from the young inflorescence is used to treat jaundice.[9] So far there is no scientific reports about this plant, hence the present study aims to evaluate the *in vitro* antioxidant and antibacterial properties and identification of phytocompounds present in leaves and fruits of *A. wightii* through RP-HPLC and GC-MS analysis.

MATERIALS AND METHODS

Sample collection, freeze drying and extraction

The leaf and fruit of *A. wightii* were freshly collected from Western Ghats, Kerala, India, which was authenticated by Dr. D. Stephen, Taxonomist, The American College, Madurai, Tamil Nadu, India. The samples were cut into small pieces and immediately frozen with liquid nitrogen and lyophilized at - 55 °C (CHRIST *Alpha* 1-2 LD plus freeze dryer, Germany) for 96 h to remove the moisture content. The powdered sample (10 g) was then extracted with 200 ml of methanol for 12-16 h with soxhlet apparatus and concentrated using rotary evaporator at 50°C. The final product of sample was stored at -20°C for further use.

Phytochemical Screening

Phytochemical screening of methanolic extract was carried out using standard protocols of Trease and Evans[10] and Harborne.[11]

Determination of total phenolics content

Total phenolics content in the plant extract was determined by Folin-Ciocalteu method Singleton and Rossi.[12] Briefly, 0.1 ml of plant extract was mixed with 0.5 ml of distilled water followed by the addition of 0.25 ml of Folin - Ciocalteu phenol reagent and allowed to stand for 6 min. Then, 0.75 ml of 20% of sodium carbonate solution was added and the final volume was made up to 3.5 ml with distilled water and the absorbance was read at 765 nm. Total phenolics content of plant extract was

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expressed as gallic acid equivalents (mg of GAE /g of plant extract).

Determination of total flavonoid content

Total flavonoid content was analyzed using modified calorimetric method of Ordon Ez et al.[13] Briefly, 0.5 ml of plant extract was mixed with 0.9 ml of distilled water and 1 ml of aluminium chloride solution. The reaction mixture was allowed to stand for 1 h at room temperature and the formation of yellow color indicates the presence of flavonoid and read at 420 nm. Total flavonoid content of plant extract was calculated as rutin equivalents (mg of RE /g of plant extract).

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The method of Liana-Pathirana and Shahidi [14] was used to determine the DPPH radical scavenging ability. One ml of DPPH solution (0.135 mM) was mixed with plant extract at different concentration and left in dark at room temperature for 30 min. Finally, the absorbance was measured at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation:

Percentage of inhibition= [(Abs control – Abs sample/ standard)] / (Abs control)] ×100

The final result was expressed as an IC_{50} value (the concentration of sample producing 50% inhibition of the DPPH free radicals; µg/ml).

2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay

ABTS radical scavenging assay was performed as described by Re et al.[15] ABTS stock solution (7 mM ABTS and 2.4 mM potassium persulphate) was prepared. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The plant extract were allowed to react with 1 ml of $ABTS^{\cdot+}$ solution after 7 min the absorbance was measured at 734 nm. The percent of ABTS free radical scavenging inhibition capacity of the extract was calculated from the following equation:

Percentage of inhibition = [(Abs control – Abs sample/ standard)] / (Abs control)] ×100

The final result was expressed as an IC_{50} value (the concentration of sample producing 50% inhibition of the ABTS free radicals; µg/ml).

Ferric reducing power assay

Ferric reducing power of the sample was measured according to the method of Oyaizu.[16] The sample with different concentrations was added with 2.5 ml of sodium phosphate buffer (pH-7.4) followed by 2.5 ml of 1% potassium ferricyanide. The reaction mixture was vortexed well and incubated at 50 °C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. To 5 ml of the supernatant, 5 ml of deionized water was added with 1 ml of 1% ferric chloride and incubated at 35°C for 10 min. The absorbance was read at 700 nm.

Nitric oxide radical scavenging assay

Nitric oxide radical scavenging activity of *A. wightii* extract was determined using the method of Garratt.[17] The reaction mixture (3 ml) contained 2 ml of 10 mM sodium nitroprusside, 0.5 ml of phosphate buffered saline (pH - 7.4) and 1 ml of plant extract and incubated for 150 min at 25 °C. After incubation, 1 ml of sulfanilamide (0.33% in 20% glacial acetic acid) was added to 0.5 ml of the incubated solution and allowed to stand for 5 min. Then 1 ml of 0.1% of napthyl ethylenediamine dihydrochloride (NED) (w/v) was added and the mixture was incubated for 30 min at room temperature. The absorbance of the chromophore that formed during diazotization of nitrite with sulfanilamide and subsequent coupling with NED was immediately recorded at 540 nm against the blank sample. Percentage inhibition was calculated as Nitric oxide radical scavenging activity (%) = (A control –A sample / A control) x 100.

The final result was expressed as an IC_{50} value (the concentration of sample producing 50% inhibition of the nitric oxide free radicals; µg/ml).

Lipid peroxidation assay

A modified thiobarbituric acid reactive species (TBARS) assay[18] was used to measure the lipid peroxide formed using egg yolk homogenates as lipid rich media.[19] To the 0.1 ml of extract, 0.5 ml of egg homogenate (10% v/v) was added and made up the volume to 1 ml with distilled water. To the reaction mixture, 0.05 ml of ferric sulfate (0.07 M) was added to induce lipid peroxidation and incubated for 30 min at room temperature. Then, 1.5 ml of 20% acetic acid (pH -3.5) and 1.5 ml of 0.8% (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulfate was added and the resulting mixture was vortexed and then heated at 95 ºC for 60 min. After cooling, 5.0 ml of butan-1-ol were added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured using spectrophotometrically at 532 nm. Inhibition of lipid peroxidation (%) by the extract was calculated.

Antimicrobial activity

Test organisms

Escherichia coli (MTCC 739), *Pseudomonas aeruginosa* (MTCC 1934), *Aeromonas hydrophila* (MTCC 1739), *Rhodococcus rhodochrous* (MTCC 265), Staphylococcus *sp.* (MTCC 2940), *Staphylococcus aureus* (MTCC 96), *Candida albicans* (MTCC 227) were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh.

Disc diffusion method

Sensitivity of test bacterial strains to methanolic extract of *A. wightii* was measured by means of zone of inhibition

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using disc diffusion assay.[20] Stock solution (10 mg/ ml) of each extract was prepared. The Mueller Hinton agar plates were prepared and standard inoculum suspensions were swabbed over the surface of the media using sterile cotton swab to ensure confluent growth of the organism. The plain sterile discs (6 mm) were placed on the inoculated Mueller Hinton agar surface and impregnated with stock solutions at (300 and 500 µg/disc). An antibiotic disc of chloramphenicol (30 mcg/disc) was used as control. The plates were incubated at 37 ºC for 24 h and the zone of inhibition was measured in milli meter (mm).

FT-IR analysis

Fourier transform infrared spectroscopy (FT-IR) was used to identify the characteristic functional groups in the plant extract. Plant extract (10 mg) was encapsulated in 100 mg of potassium bromide (KBr) pellet, in order to prepare translucent sample discs. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The powdered plant sample in each sample cup was treated for FT-IR spectroscopy (Perkin Elmer 2000 infrared spectrometer). The scan range set was from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

Conventional Assistant Extraction

A. wightii leaf power (0.5 g) mixed with 40 ml of 70% methanol then 10 ml of 6 M HCl was added. The extraction mixture was refluxed in water bath at 90 °C for 2 h. Then the extract was allowed to cool and filtered with 0.45 µm membrane filter (Pall, Bioscience, USA) prior to injection into RP-HPLC.

Analyses of phenolic compounds by RP-HPLC

The phenolic compounds of the leaf of *A. wightii* were identified by RP- HPLC based on the method described by Proestos et al. [21] with some modification. Phenolic standards used were gallic acid, ascorbic acid, chlorogenic acid, caffeine monohydrate, vanillin, o- coumaric acid and protocatechuic acid. The analytical HPLC system employed consists of high performance liquid chromatography (Waters, USA) coupled with a photodiode array detector (PDA-2998, USA). A C_{18} reverse phase column of 4.6×250 mm, 5 µm particle size (SYMMETRY) was used. The mobile phase used was water (solvent A) with 0.1% formic acid and 100% acetonitrile (solvent B). The gradient program followed for separation of phenolic compound was 0–10% B (10 min), 10–15% B (10 min), 15–20% B (5 min), 20–30% B (5 min) and 30–40% B (5 min), with 1.0 ml/ min as the flow rate, and 20 µl as the injection volume. The phenolic compounds were detected at the range of 210 - 400 nm. The data was analyzed using EMPOWER 2 data processing software from Waters (USA).

Sample derivatization and GC-MS analysis

A 100 µl of bis trimethylsilyl acetamide (BSTFA) was mixed with 100 µl of plant extract, then 20 µl of pyridine was added. This solution was incubated for 60 min at 75°C finally it was injected in to GC-MS. The methanolic extract of *A. wightii* was quantitatively performed by GC-170 MS (*Shimadzu QP 2010 PLUS system, Japan*) equipped with a capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Split less injection was performed with a purge time of 1 min. The carrier gas was helium at a flow rate of 1 ml/min. The column temperature was maintained at 50 °C for 3 minutes, then programmed at 5 °C/min to 80 °C and then at 10 °C/min to 340 °C. The inlet temperature was 280 °C, the detector temperature was 360 °C and the solvent delay was 4 min. The identification of the peaks was based on computer matching of the mass spectra with WILLY.8 LIB and NIST05s.LIB library and by direct comparison with published data.[22]

Statistical analysis

Experimental results were expressed as mean ± SD of three parallel measurements. The results were processed using Microsoft Excel 2007 and Origin 6.0.

RESULTS

The methanol extract of *A. wightii* leaf and fruit revealed the presence of phytochemicals such as phenolics, alkaloids, flavonoids, reducing sugars, saponins, steroids and terpenoids (Table 1). This study showed that total phenolics content of leaf as 11.63 ± 0.02 mg of GAE/ g, and fruit as 16.20 ± 0.60 mg of GAE/ g, and total flavonoid content of both leaf and fruit extract was found to be 13.55 ± 0.44 mg of RE/g and 7.80 ± 0.72 mg of RE/g of extract (Table 2).

Figure 1a shows DPPH radical scavenging activity of the methanolic extract of the leaf and fruit of *A. wightii* compared with BHT. It was observed that fruit extract showed higher activity than that of the leaf. At a concentration of 250 µg/ml, the scavenging activity of the leaf reached 64.72%, while at the same concentration; the fruit extract was 56.89%. The methanol extract of the leaf and fruit was found to be an effective scavenger of ABTS radical with the inhibition percentage of 94.59% and 91.89% respectively which is comparable with the standard BHT (94.89%) (Figure 1b). The present study reveals that the methanolic extract of leaf and fruit shown moderate reducing power ability with the OD value of 0.561 and 0.628 when compared to the standard BHT (1.949) at 700 nm (Figure 1c). Similarly nitric oxide radical scavenging activity was also observed to possess moderate activity in leaf and fruit (51.17% and 55.8%) at the concentrations of 250 µg/ml respectively (Figure 1d). The effect of leaf and fruit extract of *A. wightii* on peroxidation of lipids was shown in (Figure 1e). The results revealed that the fruit extract of *A. wightii* has registered the highest lipid peroxidation scavenging activity 61.8% while the leaf extract showed moderate activity of 53.89% at the concentration of 250 µg/ml compared to the standard BHT (82.62%).

The methanolic extract of *A. wightii* resulted in varying zone of inhibition (7-20 mm) for all the tested microbial pathogens (Figure 2). Leaf and fruit extract showed

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maximum activity of about 18 mm and 20 mm diameter zone against *S. aureus*. Leaf extract showed the minimum zone of inhibition 8 and 7 mm respectively against *P. aeruginosa* and *R. Rhodochrous*. Fruit showed the minimum zone of inhibition (7 mm) at the concentration of 30 µg/disc against *Staphylococcus sp.* Ciprofloxacin (30 mcg/disc) was used as standard antibiotic drug for this bio assay.

FTIR spectroscopy was used to identify the functional groups of compounds under IR region. *A. wightii* extract was passed through the FTIR in the range of 400 - 4000 cm^{-1}. The functional groups of the compounds were separated based on its peaks fraction. The FTIR results confirmed the presence of polyphenols, alcohol, alkanes, alkenes, aldehyde, aromatic compound, secondary alcohols, aromatic amines (Figure 3a and b). Fig. 4a, b and c shows the identified phenolic compounds using RP-HPLC method in the leaf extract. The leaf extract of *A. wightii* shows the presence of gallic acid, ascorbic acid, chlorogenic acid, and caffeine (Table 3) which was compared to that of the standards based on the retention time(T_R in min) and maximum absorbance(λ_{max} in nm).

Table 1: Phytochemical screening of *A. wightii* methanolic extract.

Test	Leaf	Fruit
Phenolics	++	++
Alkaloids	++	+
Flavonoids	+	+
Reducing sugars	+	++
Saponins	++	++
Steroids	++	++
Terpenoids	++	+

+ - moderate amount, **++** - appreciable amount

Table 2: Determination of total phenolic and flavonoid content of *A. wightii* methanolic extract.

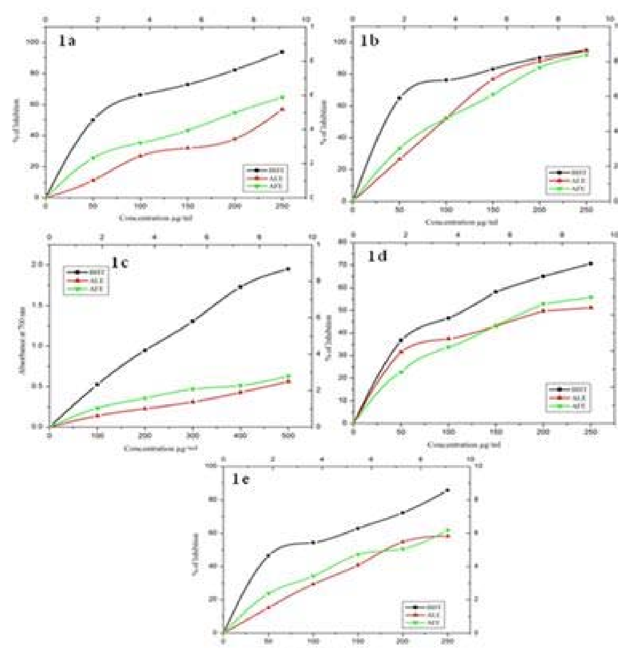
Plant Extracts	Total Phenols (mg of GAE/g dw)*	Total Flavonoids (mg of RE/g dw)*
Leaf	11.63 ± 0.02	13.55 ± 0.44
Fruit	16.20 ± 0.60	7.80 ± 0.72

*Data are presented as the mean ± standard deviation of three determinations; GAE/g dw – gallic acid equivalent/g dry weight; RE/g dw – rutin equivalent/g dry weight.

Table 3: Phenolic standards analyzed by RP-HPLC

Peak	T_R [a] (min)	λ_{max} [b] (nm)	Phenolic compound
1	1.604	210, 270	gallic acid
2	2.318	265	ascorbic acid
3	2.929	245,323	chlorogenic acid
4	3.796	210, 270	caffeine monohydrate
5	10.210	272	vanillin
6	17.075	320	O- coumaric acid
7	18.370	256	protocatechuic acid

a - Retention time (min); b - maximum absorbance (nm)



ALE- *A.wightii* leaf methanol extract; AFE- *A. wightii* fruit methanol extract; BHT-standard

Figure 1: Determination of antioxidant activity of methanolic extract of *A. wightii*. (1a) DPPH radical scavenging activity; (1b) ABTS radical scavenging activity; (1c) reducing power activity (1d) Nitric oxide scavenging activity; (1e) Lipid peroxide radical scavenging activity.

Table 4: Phytocompounds identified in A. *wightii* methanolic extract by GC-MS analysis.

(4a) Leaf extract

Peak	RT[a]	Area%	MF[b]	MW[c]	Compound Name[d]
1	11.161	85.41	C9H13N	135	Benzene ethanamine
2	14.372	4.02	$C_{10}H_{18}O_2$	170	3-Nonenoic Acid, methyl ester
3	21.277	0.64	$C_6H_{12}O_2$	116	Butanoic Acid, 3-methyl-, methyl ester
4	23.762	3.14	C8H6O4	166	Phthalic acid
5	26.244	6.80	$C_{20}H_{38}O_2$	310	13-Docosenoic acid, methyl ester

(4b) Fruit extract

Peak	RT[a]	Area%	MF[b]	MW[c]	Compound Name[d]
1	10.819	0.03	$C_5H_{10}Cl_2$	140	Pentamethylene dichloride
2	11.159	90.46	C9H13N	135	Benzene ethanamine
3	18.603	1.28	$C_4H_8O_3$	104	2-Hydroxyisobutyric acid
4	21.273	0.39	$C_6H_{12}O_2$	116	Butanoic acid, 3-methyl-, methyl ester
5	23.758	3.37	$C_8H_6O_4$	166	Phthalic Acid
6	26.209	4.48	$C_{23}H_{44}O_2$	352	13-Docosenoic acid, methyl ester

a - Retention time (as min); b - Molecular formula; c - Molecular weight; d - Compounds listed in order of retention time.

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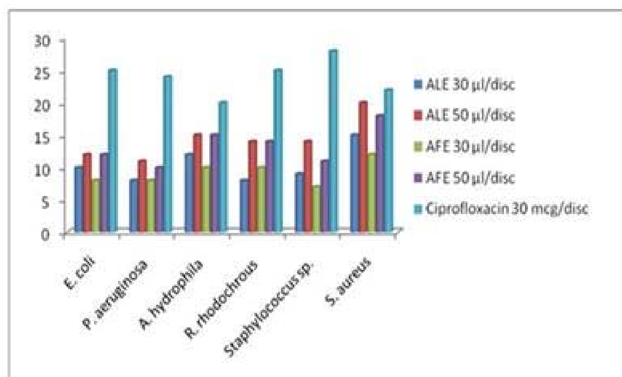

ALE- *A.wightii* leaf methanol extract; AFE- *A.wightii* fruit methanol extract; Ciprofloxacin-standard

Figure 2: Antimicrobial activity of *A. wightii* methanolic extract. Zone of inhibition in millimeter (mm).

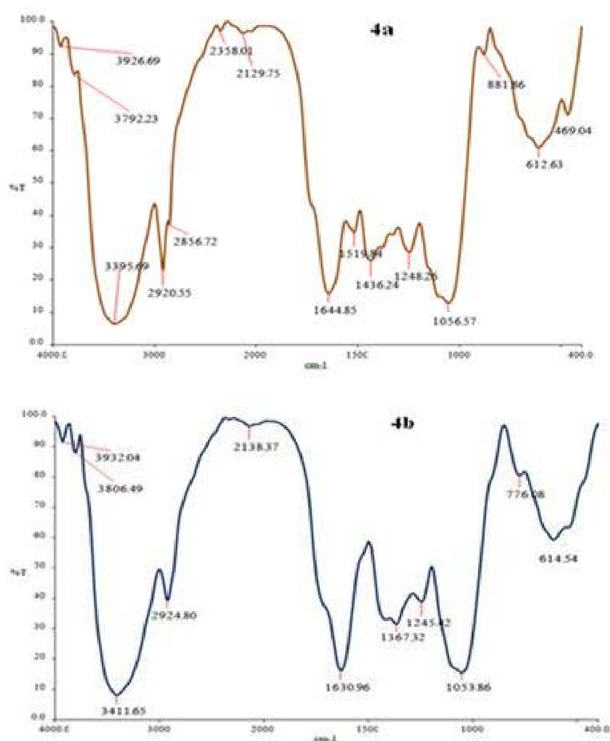

Figure 3: FT-IR profile of: (a) leaf (b) fruit sample of *A. wightii.*

Gas chromatogram of leaves and fruit extract of *A. wightii* extract is presented in Table 4a & b. A total of 11 compounds were identified in leaf and fruit extract. Benzene ethanalamine (85.41% and 90.46%), 13-docosenoic acid, methyl ester (6.80% and 4.48%), phthalic acid (3.14% and 3.37%) were identified as the major chemical constituents in the leaf and fruit extract. Other phyto compounds like 3-nonenoic acid, butanoic acid, pentamethylene dichloride and 2-hydroxyisobutyric acid also identified. The present results revealed that the methanolic extract of *A. wightii* is predominantly composed of phenolic acids and saturated fatty acids.

DISCUSSION

Polyphenols present in a variety of plants that is used as a significant components in human.[23] The results clearly show the presence of polyphenolic compounds which is well known for its antioxidant potential.[24] The antioxidant potential of phenolic compounds may be due to their redox property that allows them to act as hydrogen donars and oxygen quenchers.[25] Flavonoid, tannins and phenolic acids can be used as important indicators of the antioxidant capacity for any product that is intended to be considered as a natural source of antioxidants in functional foods.[26]

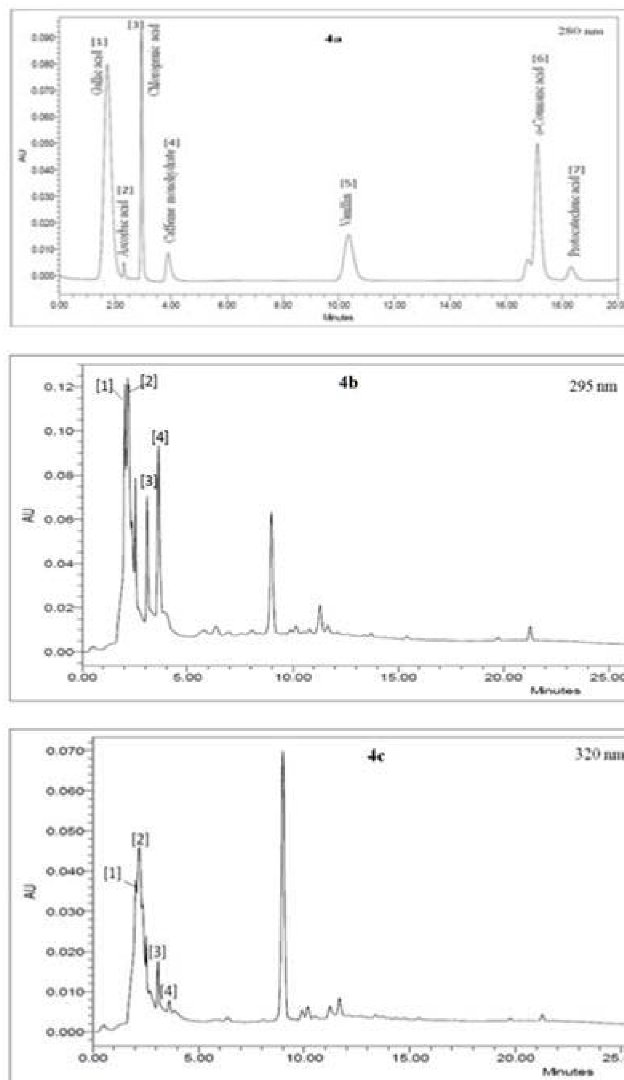

Figure 4: RP- HPLC chromatogram of phenolic compounds (a) phenolic standards (280 nm), (b) *A.wightii* leaf extract (295 nm), (c) *A.wightii* leaf extract (320 nm).

In the recent era, many research reports suggest that the pharmacological activities of medicinal plant extract mainly due to the presence of phenolic and flavonoid compounds.[27-29] The good antioxidant potential of *A. wightii* in the present study may be attributed to several reasons such as inhibition of ferryl-perferyl complex formation; OH radical or nitric oxide radical scavenging; reducing the rate of conversions of Fe^{3+} to Fe^{2+} or by chelating of the iron itself.[30] DPPH is a stable synthetic free radical that accepts an electron or hydrogen radical to become a stable molecule.[31, 32] DPPH radical is widely used to evaluate the antioxidant activity since because it

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is sensitive enough to detect active ingredient at low concentration. In the present investigations, *A. wightii* exhibited a concentration dependent antiradical potential by quenching DPPH radical. Reducing power assay was evaluated by the measuring the transformation of Fe (III) to Fe (II) in the presence of plant methanolic extract. The ability to reduce Fe (III) may be attributed to the hydrogen donating effect of bioactive compounds present in the plant. Natural antioxidants reduce the Fe^{3+} to Fe^{2+} form by donating an electron.[33, 34] Reductones present in the extract also play a vital role in reducing power[35], which donates a hydrogen atom and breaks the free radical chain.[36]

Nitric oxide is a free radicals generated in mammalian cells which is engaged in the regulation of various physiological processes. Even then, excess production of NO is associated with several diseases. In our present study, the nitrite produced by the incubation of sodium nitroprusside in phosphate buffer was significantly reduced by the sample. The inhibition of generation of nitrite in the solution may be due to the antioxidant principles in the extract that compete with O_2 to react with nitric oxide.[37] Similarly, lipid peroxidation is the peroxidation of polyunsaturated fatty acid in the cell membranes forming melondialdehyde (MDA) as the bye product due to the presence of numerous carbon– carbon double bonds.[38] Since, unsaturated fatty acids are unstable, they can easily react with reactive oxygen species (ROS) to form lipid peroxide radicals which cause cellular damage requiring DNA repair.[39] Egg yolk phospholipids demonstrated rapid non-enzymatic peroxidation on incubation with ferrous sulphate and the efficacy of lipid peroxidation scavenging activity of methanolic extract of *A. wightii* was comparable with that of the standard BHT.

Plant natural products like flavonoids, phenolics and fatty acids have been proved to have strong antioxidant and antimicrobial activity.[40] In natural system, treatment of human and animal diseases using plant extracts has long been trained before the initiation of antibiotics.[41,42] Previous report of Dryden [43] suggests that *S. aureus* is the most common skin infectious pathogen throughout the world. Similarly the leaf extract of *A. ornata* against bacteria like *S. aureus, P. aeruginosa*, supports its traditional medicinal value.[44] Phytochemicals such as phenolics and flavanoids are important secondary metabolites that are generally present in leaves, vegetables, fruits and cereal grains. These compounds are natural; act as antioxidants and the main role of these secondary metabolites is to defend against degenerative diseases, cardiovascular diseases, cancer and aging.[45] The current study also shows the presence of active phenolic compounds such as gallic acid, ascorbic acid, chlorogenic acid and caffeine. Phenolic acids and its derivatives have been present in many phytomedicines with a number of biological and pharmacological activities, including anti microbial and free radicals scavenging activity. Moreover, GC-MS analysis shows the presence of phenolics, fatty acids, aromatic, aliphatic and other organic acids in the plant samples. Volatile compounds and saturated and unsaturated fatty acids were also identified in date palm (*P. dactylifera* L.) extract.[46] Considering these factors we suggest that the biological activity of plant extract might be due to the presence of these compounds in the methanolic extract of leaf and fruit of *A. wightii*.

CONCLUSION

The results obtained in the present study reports the antioxidant and antimicrobial activity of *A. wightii* extract. Antibacterial activity of both the extract (leaf and fruit) has the significant activity against the human pathogenic organism *S. aureus*. The examined antimicrobial activity confirms the valuable traditional use of this herbal drug against the microbes. RP- HPLC analysis has detected four major phenolic compounds (gallic acid, ascorbic acid, chlorogenic acid, and caffeine). Both HPLC and GC–MS analysis of methanolic extract of *A. wightii* reveals the presence of antioxidant and antimicrobial phytochemicals. Further investigation is under conduction to explore the other poly phenolic compounds through LC-MS/MS analysis from the extract of *A. wightii*. Hence, we suggest that the leaves and fruits of *A. wightii* will be a source of natural products with potential use against pathogenic microbes in the pharmaceutical industry.

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