P. Arulpriya et al. / Journal of Pharmacy Research 2014,8(7),893-898

Research Article
ISSN: 0974-6943

Available online through
http://jprsolutions.info

Anti-inflammatory and antioxidant activity index of the aerial roots of *Rhaphidophora aurea* (Linden Ex Andre) intertwined over different host trees

P. Arulpriya and P.Lalitha*
Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore- 641043, Tamil Nadu, India.

Received on:21-04-2014; Revised on: 17-05-2014; Accepted on:25-06-2014

ABSTRACT

Background: *Rhaphidophora aurea* is a common ornamental plant with aerial roots required for its support. It climbs over host trees and sucks the nutrients of the host. A similar species *Epiprenum pinnatum* is reported to possess anticancer activity. The leaves of *Rhaphidophora aurea* are toxic but there are no reports on the uses of aerial roots. The weird property of the aerial roots to twine over the host and suck its nutrients persuaded us to explore the properties of the host and uncover the acquired therapeutic properties of the aerial roots of *Rhaphidophora aurea*. **Materials and methods:** The antioxidant activity index of various solvent extracts of the aerial roots of *Rhaphidophora aurea* intertwined over four different host trees were evaluated by DPPH radical scavenging method and reducing power assay. IC_{50}, EC_{50} and antioxidant activity index (AAI) were taken up for antioxidant assessment. The ethyl alcohol extract of the aerial roots of *Rhaphidophora aurea* twined over *Lawsonia inermis* (MM) and *Areca catechu* (MB) were tested for anti-inflammatory activity in formalin induced Swiss Albino mice paw oedema model and the paw thickness was measured each one hour upto six hours. **Results and Conclusions:** Among all the extracts evaluated, the ethyl alcohol extract of the aerial roots of *Rhaphidophora aurea* twined over four host trees specially *Azadirachta indica* (MN) exhibit higher antioxidant activity with a strong AAI (5.29) as compared to AAI (2.74) of standard Ascorbic acid. In formalin induced inflammation model, the dose (100mg/kg) produced significant percentage inhibition of mice paw oedema compared to control group. It can be concluded that alcoholic extract of aerial roots of *Rhaphidophora aurea* intertwined over four different host trees shows good *in vitro* antioxidant and *in vivo* anti-inflammatory activity and hence of good promise in pharmaceutical field.

Keywords: *Rhaphidophora aurea*, Antioxidant activity index, DPPH radical scavenging, Reducing power assay, Anti-inflammatory activity.

INTRODUCTION

Oxygen free radicals bring about damage of tissues due to peroxidation of biomembranes.[1] Free radicals are produced due to respiration, by radiation, microbes, alcohol, smoking, emotional stress,[2] ecological poisonous substance, medicines and pathogens. Oxidative damage in cell leads to dangerous etiological features implicated in abundant chronic disorder in human such as cancer, diabetes, arthritis, atherosclerosis, neurodegenerative diseases, ageing,[3] decreased membrane fluidity, ischemic heart disease,[4] anemia, asthma, inflammation, Parkinson diseases, Mongolism and stroke.

Mechanism of inflammation is attributed by reactive oxygen species (ROS) generated from activated neutrophils and macrophages. The over production of ROS will result in tissue injury by damaging macromolecules and lipid per oxidation of membranes. Additionally it propagates inflammation, by stimulating release of cytokines such as IL-1, TNF- α and interferon-γ that are responsible for the additional neutrophils and macrophages. Hence free radicals are significant mediators that provoke or prolong inflammatory responses and its neutralization by antioxidants and radical scavengers will reduce inflammation [6,7].

Plants are rich sources of natural products and enclosed traditional medicinal values, because they are contain rich source of antioxidant property in it. Antioxidants derived from plants quench the free radicals by contributing hydrogen[5]. Plant constituents like flavonoids, phenols, ascorbic acid, polyphenols, sterols and glycosides exhibit notable antioxidant activity and possess pharmacological properties. Antioxidant compounds from plant extracts perform by free radical scavenging, oxygen quenching, chelation of transitional metals, reducing agents to restrain radical damage in biological organisms.

The plant *Rhaphidophora aurea* (Linden ex Andre) is often described as money plant. The aerial roots have a special characteristic to grow

***Corresponding author.**
Dr. P.Lalitha,
Assistant Professor (SG) of Chemistry,
Avinashilingam Institute for Home Science and Higher Education for Women,
Coimbatore- 641043, Tamil Nadu, India.

by sucking nutrients from host trees on which it intertwines. The solvent extracts of *Epipremnum aureum* (Araceae) exhibits antitermite, antibacterial, antioxidant property[8] and *Epipremnum pinnatum* exhibit anticancer activity[9]. Due to the unique nature of this plant, the extracts of the aerial roots of *Rhaphidophora aurea* twined over different host trees were taken up to estimate its antioxidant and inflammatory potential.

MATERIALS AND METHODS

Plant material

The aerial roots of *Rhaphidophora aurea* intertwined over *Lawsonia inermis* (MM) and *Azadirachta indica* (MN) were collected from Coimbatore District; roots twined over *Areca catechu* (MB) and *Cocos nucifera* (MC) were collected from Palakkad District. Sequential extractions of the aerial roots were carried out by refluxing (12 hours) with appropriate volume of solvents and the process was repeated until the pale colour or colourless of solvent was noted. The solvents were filtered and distilled using a flash evaporator to get the extracts.

Phytochemical screening

The phytochemical screening of solvents and aqueous extract of MM, MB, MC and MN were carried out using standard procedures[10], to identify the constituents like alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides, anthraquinons, phenolics, carbohydrates, quinines and reducing sugars.

Antioxidant assay

The petroleum ether, ethyl acetate, ethanol and aqueous extract of MM, MN, MC and MN were evaluated for antioxidant activity by DPPH radical scavenging and reducing power assay and the results compared with Ascorbic acid. Solvent and aqueous extracts codes used in this paper are indicated below:

Solvents	PubChem CID	MM	MB	MC	MN
Petroleum ether	-	M1	B1	C1	N1
Ethyl acetate	8857	M2	B2	C2	N2
Ethanol	702	M3	B3	C3	N3
Aqueous	-	M4	B4	C4	N4

Preparation of stock solution

The extracts and Ascorbic acid were weighed (1 mg) and made up to 10 ml with distilled methanol. Various concentrations (10, 20, 30, 40, 50 and 60 µg/ml) were prepared for the study by appropriate dilution of the stock solution.

DPPH radical scavenging assay

The hydrogen donor activities of the extracts were measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) [PubChem CID 74358](Blois method, 1958) assay. [11] 2ml of 0.1Mm DPPH (methanolic solution) was added to various concentrations of extract (1 ml) and the absorbance was measured in a colorimeter at 517nm. The percentage of antioxidant activity was calculated as

% of DPPH scavenged = B_0- [B_1/B_0] ×100

Where, B_0= Absorbance of the control, B_1= Absorbance of the sample

Reducing power assay

The reducing power ability of the extract was determined by Oyaizu method.[12] The extract (1 ml) was added to 2.5 ml of phosphate buffer (0.2M monobasic sodium phosphate, 0.2M dibasic sodium phosphate) and 2.5 ml of one percent potassium ferricyanide. The mixture was incubated at 50ºC for 20 min. Then 2.5 ml of 10% (TCA) Trichloroacetic acid was added to the mixture and centrifuged. The supernatant (1ml) was treated with three ml of purified water and 0.5 ml ($FeCl_3$) of ferric chloride. Absorbance value of both the extracts and standard was measured at 700nm.

Determination of IC_{50} and EC_{50}

Masterplex 2010 software was used to calculate the half maximal inhibitory concentration, effective concentration and linear regression analysis.

Antioxidant activity index (AAI)

The antioxidant activity index (AAI) was calculated by the IC_{50} value of the extract and standard (Scherer and Godoy). [13, 14]

AAI= DPPH (µg/ml)/ IC_{50} (µg/ml)

Anti- inflammatory studies

Animals

Healthy young Swiss Albino male mice (18-32g) were kept in groups of 3-4 per cage. The protocol of these experiments was approved by the Ethical committee of KMCH College of Pharmacy, Coimbatore-48. The authentication number is KMCRET/PhD03/2010-11.

Housing and feeding

The animals were housed in polypropylene cages at room temperature. The standard laboratory animal food pellets with water *ad libitum* was supplied to animals during the study period.

Anti-inflammatory studies

The study was carried out by adopting the procedure of Nuhu et al., 2010[15] with slight modifications. Swiss Albino mice were divided into four groups of 4 mice each. The groups were treated intraperitoneally; group 1 received 10 mg of ketoprofen per kg (+ve control), group 2 received 1ml normal saline per kg (-ve control), group 3 received 100mg/kg plant extract of MM and group 4 received 100 mg of plant product extract of MB per kg body weight respectively. After thirty minutes, formalin was injected to all the four group mice and the

difference in diameter of the right paw and left hind paw was measured using a Vernier caliper at the regular interval of 1 hour up to six hours. The percentage inhibition of the expansion of oedema was calculated as

$$\% \text{ inhibition} = \frac{(St\text{-}Sc)\ \text{control} - (St\text{-}Sc)\ \text{treated}}{(St\text{-}Sc)\ \text{control}} \times 100$$

St-the mean paw size for each group after treatment
Sc-the mean paw size obtained for each group before injection

Statistical analysis

All measurements in this study were recorded as mean ± standard error of the mean (SEM) and statistical analysis was done using One way ANOVA using Dunnett's test Values with $P < 0.05$ considered as being significant.

RESULTS AND DISCUSSION

Antioxidants protect oxidative damage originated by the free radicals in many ways. Numerous antioxidant evaluation assays have been developed to identify the resolution capacity of plant antioxidant and these *in vitro* assays give a valuable measure of the potential of plants. In this present study DPPH radical scavenging and reducing power assay have been chosen to determine the antioxidant activity of the extracts of the aerial roots of *Rhaphidophora aurea* twined over various host trees.

DPPH radical scavenging activity

DPPH is a stable free radical at room temperature and adopt an electron to become a stable diamagnetic fragment. The reduction potential of DPPH radical was determined by the increasing percentage inhibition.

The DPPH test provides information about the reactivity of test compounds with a stable free radical which possess odd electron and gives a strong absorption at 517 nm in deep violet color. Due to the simplicity and convenience of the method, this assay is gaining attention.

The results of percentage inhibition of DPPH assay of aerial roots of *Rhaphidophora aurea* twined over MM, MB, MC and MN are given in the figure 1. The results clearly show that the ethanol extract of MM, MB, MC and MN exhibit maximum percentage inhibition at 60µg concentration. The antioxidant percentage inhibition/absorbance indications are given table 1.

Based on the table 1 indication, ascorbic acid, M1, M2, M3, B1, B2, B3, C2, C3, N1, and N3 are found to posses higher antioxidant activity and M4, B4, C1, C4, N2 N4 medium antioxidant activity. The extract N3 exhibited extremely remarkable high antioxidant power compared to that of ascorbic acid.

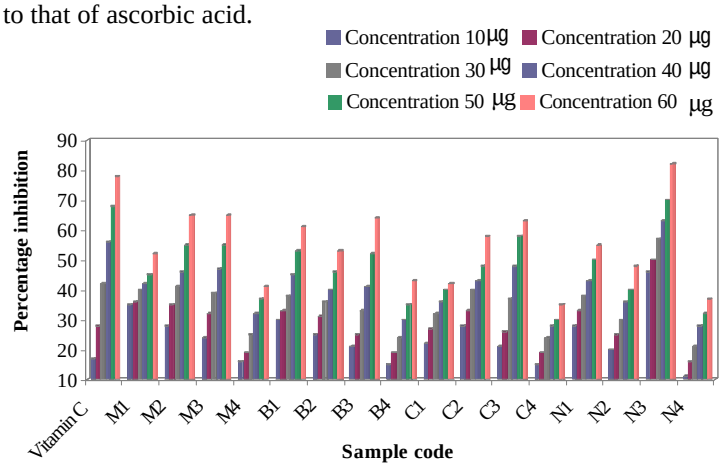

Figure 1: DPPH percentage inhibition of aerial roots of *Rhaphidophora aurea* twined over MM, MB, MC and MN

Table 1: Antioxidant activity (AA) percentage inhibition/absorbance indication[16]

AA< 25% inhibition AA< 0.25 absorbance	Low antioxidant power
AA 25-50% inhibition AA< 0.25-0.50 absorbance	Medium antioxidant power
AA 50-80% inhibition AA< 0.50-0.80 absorbance	High antioxidant power
AAI > 80% inhibition AA< 0.80 absorbance	Extremely high antioxidant power

The AAI indications and half maximal inhibitory concentration and antioxidant activity index (AAI) extracts results are given in table 2 and 3.

Table 2: Antioxidant activity index value indication[13]

AAI <0.5	Poor antioxidant activity
AAI between 0.5 and 1.0	Moderate antioxidant activity
AAI between 1.0 and 2.0	Strong antioxidant activity
AAI > 2.0	Very strong antioxidant activity

From table 3 the half maximal inhibitory concentration of N3 is found to be 18.87µg/ml as compared to Ascorbic acid (36.46µg/ml). The other extract concentrations showed activity comparable to that of standard. N3 exhibited very strong antioxidant activity which may be due to the presence of phytoconstituents like alkaloid, flavonoid, anthocyanins, antraquinones .[32] The seed extracts of *Azadirachta indica*, [34] ethanolic leaf extract of *Azadirachta indica*,[33] ethanol, methanol, hexane, butanol and water extract of bark and leafs of

Table 3: IC_{50} and AAI of aerial roots of *Rhaphidophora aurea* twined over MM, MB, MC and MN

Sample Code	Regression Equation	R^2	IC_{50} (µg/ml)	AAI
Ascorbic acid	y=1.2542x+4.2666	0.9972	36.46	2.74
M1	y=0.3257x+30.2666	0.9408	60.58	1.65
M2	y=0.7142x+20.0000	0.9854	42.00	2.38
M3	y=0.8057x+15.4666	0.9971	42.86	2.33
M4	y=0.5314x+9.7333	0.9897	75.77	1.31
B1	y=0.6342x+21.1333	0.976	45.51	2.19
B2	y=0.5400x+19.6000	0.9937	56.29	1.77
B3	y=0.8685x+8.9333	0.9755	47.28	2.11
B4	y=0.5542x+8.2666	0.9895	75.3	1.32
C1	y=0.4085x+18.8666	0.9841	76.21	1.31
C2	y=0.5657x+21.8666	0.9768	79.73	1.25
C3	y=0.9057x+10.4666	0.9867	43.64	2.29
C4	y=0.3914x+11.4666	0.99	98.45	1.01
N1	y=0.5457x+22.0666	0.9967	51.18	1.95
N2	y=0.5457x+14.0666	0.9929	65.84	1.51
N3	y=0.7028x+36.7333	0.9742	18.87	5.29
N4	y=0.5285x+5.6666	0.9961	83.88	1.19

Azadirachta indica [35] are reported to show strong antioxidant activity. Strong activity of M2, M3, B1, B3 and C3 may be due to the presence of constituents like flavonoids, anthocyanins, glycosides and phenols [32] in it. Hence the strong and very strong activity of the extracts may be contributed to the phytoconstituents and nature of host tree.

Reducing power assay

Substances, which have reduction potential, respond with Fe^{3+} to form Fe^{2+}, which then react with ferric chloride to form ferric ferrous complex that has absorbance maximum at 700nm. Higher absorbance indicates higher antioxidant power (Table 1). The absorbance efficiency of the extracts and half maximal effective concentration are given in figure 2 and table 4.

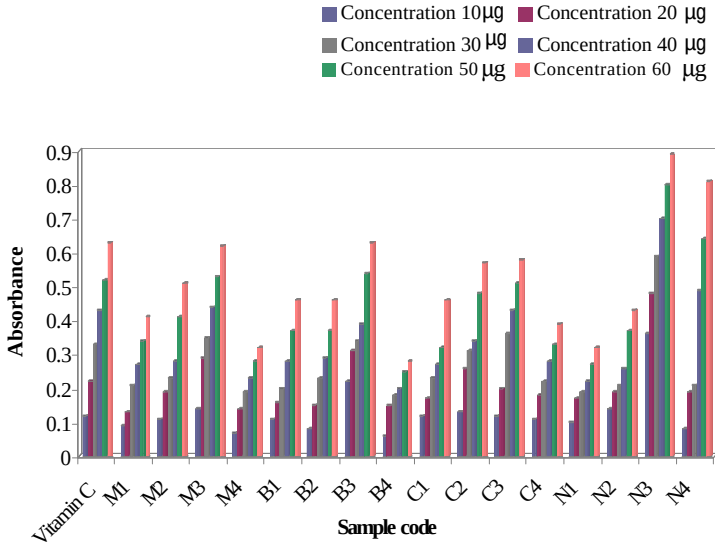

Figure 2: Reducing ability of aerial roots of *Rhaphidophora aurea* twined over MM, MB, MC and MN

From the figure 2 and table 4 it is well obvious that N3 exhibits maximum absorbance and higher antioxidant activity. The EC_{50} (22.20µg/ml) is also very less compared to other extracts and ascorbic acid. The rest of the other extracts activity was comparable to that of the standard. In general the increasing absorbance in reducing power assay can be correlated to increasing antioxidant ability.

Table 4: EC_{50} value of aerial roots of *Rhaphidophora aurea* twined over MM, MB, MC and MN

Sample Code	Maximum Absorbance (700nm)	Regression Equation	R^2	EC_{50} (µg/ml)
Ascorbic acid	0.63	y=0.0101x+0.0200	0.9993	47.52
M1	0.41	y=0.0065x+0.0126	0.9951	74.98
M2	0.51	y=0.0077x+0.0173	0.9635	62.68
M3	0.62	y=0.0091x+0.0740	0.9882	46.81
M4	0.32	y=0.0048x+0.0340	0.991	97.08
B1	0.46	y=0.0070x+0.0173	0.9764	68.95
B2	0.46	y=0.0074x+0.0013	0.9972	67.39
B3	0.63	y=0.0079x+0.1260	0.9541	47.34
B4	0.28	y=0.0040x+0.0446	0.9496	113.85
C1	0.46	y=0.0062x+0.0426	0.9478	73.77
C2	0.57	y=0.0082x+0.0593	0.9662	53.74
C3	0.58	y=0.0094x+0.0366	0.98	49.29
C4	0.39	y=0.0054x+0.0606	0.9967	81.37
N1	0.32	y=0.0040x+0.0686	0.9777	107.85
N2	0.43	y=0.0058x+0.0626	0.9507	75.41
N3	0.89	y=0.0106x+0.2646	0.9977	22.2
N4	0.81	y=0.0150x-0.1246	0.9565	41.64

Effect of concentration

The results from figures (1 and 2) and tables (3 and 4) portray the higher antioxidant activity with increasing concentration of extracts which may be due to the availability of various phytochemicals in these extracts. These results are in par with the observations of the previous work reported in literature.[17-19]

The strongest antioxidant power was exhibited by the ethanol extract of MM, MB, MC and MN which contains constituents like alkaloids, flavonoids, phenols, steroids, terpenoids, tannin, glycosides and anthocyanin. Most of these secondary constituents have a tendency to cure human disorders and reported to possess medicinal value. Notably flavonoids are descripted to possess antiviral, antimicrobial, anticarcinogenic, antioxidant, anti-inflammatory, vascular, oestrogenic, antimutagenic, antitumor activities and other natural activities. [23, 13] similarly, anthocyanin possesses antioxidant, antibacterial, anti-cancer, antifungal, anti-inflammatory and antiviral activity [13]. Phenol and poly phenolic compounds have potential to manage oxidative-damage related diseases such as cancer, anti-inflammatory, anti-microbial and coronary-artery disease.[13, 24]

Numerous studies have evaluated the relationship between the antioxidant activity and phenol content. In the present study, the findings did not illustrate any relationship between antioxidant activity

and phenolic content. The extract with high antioxidant and free radical scavenging activity did not show a high phenolic content. On the other hand low phenolic content implies that the type of phenolics is determinant of these activities rather than their amounts. The dissimilarity in antioxidant activities of plant extracts might be due to different qualitative and quantitative compositions of their phenolic components, from phenolic acids to flavonoids and their derivatives. [30, 31]

Anti-inflammatory activity

The mechanism of anti-inflammatory activity is associated with antagonistic action of tannins. The anti-inflammatory activity effects may be elicited by a variety of chemical agents and that there is no remarkable correlation between their pharmacological activity and chemical structure.[20] This fact, associated with the complexity of the inflammatory process, makes use of different experiment models essential when conducting pharmacological trial.

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. The phytochemical analysis of many medicinal plant extracts revealed the presence of triterpenoids, volatile oils, alkaloids, flavonoids, saponins and tannins. Alkaloids and flavonoids are well known for their ability to inhibit pain perception. [21] Flavonoids as anti-oxidants even have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation.[22]

The oedema produced via formalin was inhibited significantly ($P < 0.05$) by Ketoprofen, MM and MB extracts. The paw size was reduced from 0.51 ± 0.08 cm to 0.28 ± 0.05a cm (sample 1-4) at 3 hours. The result shows that the 100 mg/kg dose was more potent than the lower doses (Table 5).

Table 5: Anti-inflammatory activity of ethanol extract of MM and MB

Sample	Diameter (cm) in hours					
	T0	T1	T2	T3	T4	T5
Saline	0.67 ± 0.09	0.60 ± 0.10	0.61 ± 0.12	0.51 ± 0.08	0.36 ± 0.07	0.28 ± 0.11
Ketoprofen	0.57 ± 0.11	0.42 ± 0.11[a]	0.34 ± 0.07[a]	0.34 ± 0.13[a]	0.24 ± 0.10[a]	0.19 ± 0.08[a]
MM	0.43 ± 0.02[ab]	0.48 ± 0.06[a]	0.44 ± 0.01[a]	0.37 ± 0.02[a]	0.31 ± 0.02	0.24 ± 0.02
MB	0.49 ± 0.07[ab]	0.54 ± 0.08	0.42 ± 0.08[a]	0.28 ± 0.05[a]	0.27 ± 0.05	0.23 ± 0.03

Values are the mean ± SD, n=3, a, b, c are significant at **P < 0.05, compared to a – S1 vs S2, S3, S4; b – S2 vs S3, S4 and c – S3 & S4

Compared to all the test samples the '*P*' value was found to be significant for Ketoprofen, MM and MB at the third hour. These results prove that the ethanol extract of MM and MB possess almost equal activity when compared with test ketoprofen.

Anti-inflammatory activity of many plants has been attributed to their high sterol, triterpene[25] or flavonoid contents .[26] Studies have also demonstrated that flavonoids such as rutin, quercetin, luteolin, and triterpenoids produce significant anti-inflammatory activity.[27, 28] It is also shown that these pharmacological substances could exhibit anti-inflammatory activity through inhibition of cyclo-oxygenase lipo-oxygenase pathways.[29] In the present study MM and MB extracts exhibit high sterol, anthocyanin and flavonoid content, this may be attributed to the significant anti-inflammatory activity.

The obtained results reveal that all the solvent extracts of MM, MB, MC, MN and the ethanol extract of MM and MB possesses good antioxidant and anti-inflammatory properties.

CONCLUSION

The antioxidant evaluation confirms that, the ethyl acetate and ethanol extract of the aerial roots of *Rhaphidophora aurea* twined over four different host trees exhibit strong antioxidant activity, comparable to that of Ascorbic acid. The ethanol extract of MN exposed strong antioxidant potential, due to the synergistic effect of secondary metabolite and remedial nature of its host *Azadirachta indica*. The anti-inflammatory activity of the ethanol extract of MM and MB exposed significant inhibition. The results obtained from the antioxidant and anti-inflammatory studies have proven that the plant in particular its aerial roots has medicinal importance.

ACKNOWLEDGEMENT

The authors thank the authorities of the Avinashilingam Institute for Home Science and Higher Education for Women (Estd. u/s 3 of UGC Act 1956), Coimbatore -43 for having provided the facilities to carry out this research work. The pharmaceutical assistance of KMCH College of Pharmacy, Coimbatore-48 is acknowledged.

CONFLICTS OF INTEREST

No conflicts interest.

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Source of support: Nil , Conflict of interest: None