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ORIGINAL ARTICLE

The Comparative Evaluation of Antioxidant potential of various aerial part extracts of *Carissa carandas Linn*(C), *Nerium indicum Mill* (N). and *Wrightia tinctoria R.Br* (W).

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ABSTRACT
Since from time immortal the plants are important source of medicines due to their inherent chemistry of containing vital Constituents to fight against environmental stress. Therefore, present study was performed with an aim to evaluate and compare antioxidant potential of aerial parts (fruit, leaf, stem and flowers) extracts of three major plants viz.; Carissa carandas Linn(C), Nerium indicum Mill(N). and Wrightia tinctoria R.Br (W)., of Apocynaceae family, which is one of the important family of traditional medicinal plants. The plants extracted in ethanol, 60% ethanol and distilled water, were also screened for major phytoconstituents. Respective extracts were screened for total phenolic content (Folin Ciocalteu's reagent expressed as gallic acid equivalents, mg/g GAE), total flavonoid contents [Aluminium chloride (2%) method expressed as Rutin equivalents RE/g], in vitro antioxidant assays (using; 2, 2-diphenyl-1-picrylhydrazil (DPPH), hydrogen peroxide (H 2 O 2) and reducing power assay (RPA). Comparison of IC50 and % inhibition of the extracts against Ascorbic acid, Rutin and Gallic acid was done. The obtained results illustrated the GAE mg/g in the range of 537.0 to 240.933 and Rutin Equivalent (RE)/g from 113.6333 to 1049.7.The order of DPPH and H 2 O 2 were found as IC50 values. The observed results indicate that flavonoids and phenolic compounds correspond to enhance antioxidant potential. Hence it is concluded that Apocyanceae plants are rich source of antioxidants and thus have various applications in traditional system of medicine.
Keywords: Apocynaceae, Traditional and complementary medicine, Antioxidant screening, free radicals.

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INTRODUCTION

Free radicals are produced from the oxidation and reduction steps of normal metabolic processes of present in every cell of aerobes. The reactive oxygen species (ROS) and reactive nitrogen species (RNS) and non-reactive radical species Oxidative stress caused by higher levels of free radicals generated by mitochondria, endoplasmic reticulum, peroxisomes, golgi bodies and Phagocytes etc. can damage important biomolecules (nucleic acids, lipids, protein or other cellular structures) [1]. Several problems arise due to compensated redox status/immune balance [2]. Various neurodegenerative, Cardovascular, autoimmune, inflammatory and infectious diseases are now linked with free radicals [3].

The fruits and vegetables are rich source of antioxidants. Plant metabolites are best remedy to enhance immunity/defense. Nature is the reservoir full of therapeutic principles.

Non-enzymatic/exogenous antioxidants like phenols and Phenolics, Anthocyanin, Bioflavonoid/ Flavonols, Terpenoids, Coumarins and Vitamins (C, D, E etc.) are actively involved in the defense system of plants and other living beings [4, 5].

Traditional and complementary medicines are widely accepted therapeutics worldwide but its adoption in mainstay is still compromised. Traditional therapy is common acute illnesses prevailing among the

locals with no toxic effects. Some forms of TM such as Ayurveda, traditional Chinese medicine and Unani medicine are popular nationally, as well as being used worldwide. Most of the developing countries like South Africa, Singapore, Republic of Korea, India, Mongolia etc. has wide acceptability and faith on traditional medicines because of its affordability, availability and healing with improved immunity. There is an associated risk with traditional medicine like, poor quality, adulterated or counterfeit products, Misdiagnosis, late diagnosis or failure to use effective conventional treatment, exposure to misleading or unreliable information, less knowledge about the interactions, untoward or side effects. To provide the standard herbal therapy globally, a regulatory network of the International Regulatory Cooperation on Herbal Medicine (IRCH) is active currently [6].

An Innate and Adaptive form of Immunity makes one's body to withstand both organic and functional kinds of stress. Antioxidants boost immunity and lower the incidence of Non-communicable /communicable diseases. Non-communicable diseases (NCDs. Deaths due to cancer in India are 9% among all NCDs [7].Cancer mortality in India has doubled from 1990 to 2016.

Most of the present day anti-infective, anticancer, anthelmintic, Vector-borne diseases and cardiovascular drugs are from natural origins [8]. Aboriginals, disconnected from the central facilities are surviving even today by practicing natural medicines. Antioxidant phenols [9-14].

Apocynaceae family, also called as Dogbane or arrow poisons is the fifth valuable medicinal plant family in Angiosperms. Plants of the family like, *Rauwolfia, Icnocarpus, Alstonia, Catharanthus, Plumeria, Ichnocapus, Ochrosia* etc. has been claimed in many disease and ailments [15-18].

This study reports the antioxidant activity of *Carissa carandas* [19-22], *Nerium indicum* [23, 24], and *Wrightia tinctoria* [25-27].

In previous studies the free radical scavenging activity was reported in the individual parts of the plants and in some were the comparative studies. Roots, Leaves, flowers, bark, fruits etc. of these plants were used to assess the antioxidants. Extract was prepared mostly in ethanol, methanol or water using Soxhlet apparatus.

Here, the authors have used aerial parts of the aforementioned plants which included whole parts of the plant minus the roots. We aimed to observe the additive synergism enhancing the overall antioxidant capacity of aerial parts extracts compared to that of the individual part extracts.

Completely developed plants without derooting were utilized. Several studies have shown that unregularized exploitation of plants for medicines/ neutraceuticals etc. and established higher pollution levels has led to extinction of many valuable plants.

MATERIAL AND METHODS

Collection and authentication of plant

Parts of all plants namely *Carissa carandas*, *Nerium indicum*, and *Wrightia tinctoria*, (except root) were collected in equal quantity from Bundelkhand region (Jhansi). The Plants were identified and authenticated by Dr. Mudailiya, taxonomist NVARI, Jhansi (UP) India, and deposited herbarium specimen no. 24380, 24381 and 24382 for future reference.

Extraction

The collected aerial parts of each plant were washed, shade dried, pulverized and sieved. Conventional extraction method [28, 29] with slight modification was used to perform extraction using soxhlet apparatus [Thermo Fisher Scientific] using ethanol (solvent 1), 60% ethanol (solvent 2) and distilled water (solvent 3) as solvents. The ethanolic and hydroethanolic extracts were concentrated and dried in vacuum rotary evaporator and aqueous extracts dried using lyophilizer according to the standard guidelines [30-33]. Percentage yield was tabulated (Table 1) and stored in deep freezer for future use.

Phytoconstituents analysis

Qualitative analysis for major constituents was performed by using standard methods [Table 2] for Alkaloids, Flavonoids, Glycosides, Terpenoids, Carbohydrates, Saponins, Tannins, Phenolic compounds [34-39].

Quantitative estimation was performed for Phenolic compounds and Flavonoids using methods adopted widely [Table 2, Fig. 4].

Total Flavonoid Content Estimation [40]

Different concentrations of Rutin (20, 40, 60, 80 and 100 µg/ml) in methanol and test sample in methanol (100 µg/ml) were prepared. 0.5-mL aliquot of appropriately diluted sample solution with 2 mL of distilled water was mixed and subsequently with 0.15 ml of a 5% $NaNO_2$ solution. After 6 min, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to the mixture. Water was immediately, added to bring the final volume to 5 ml, and then after thoroughly mixing the mixture, it was allowed to stand for another 15 min. Absorbance of this mixture is

obtained at 510 nm versus prepared water blank. Put absorbance of test sample in line of regression of standard curve of Rutin (Fig. 2) and the total flavonoid content were calculated. The value expressed as mg/gm or, µg/mg Rutin equivalent (RE) [Fig. 2].

Alkaloid Content Estimation [41]

For the calibration curve, the concentration of 5 was used. 100ppm solution was initially prepared from atropine (1 mg in 10 ml of distilled water) and then dividing by 5 to funnel removed and, respectively, 20, 40, 60, 80 and 100 µg/ml atropine was added. The plant extract (1mg/ml) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. 1 ml of this solution was transferred to a separating funnel and then 5 ml of Bromocresol green solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. Line of regression from atropine was used for estimation of unknown alkaloid content. From standard curve of atropine, line of regression was found to be:

$y = 0.001x - 0.102$ and $R2 = 0.981$

Thus the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned atropine [Fig. 3].

Total Phenolic Content Estimation [42-45]

Different concentrations of Gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in methanol (0.1and 1mg/ml). 0.5 ml of different concentrations of Gallic acid/ test sample was added with 2 ml Folin-Ciocalteu Reagent (1:10 in deionized water), then to this 4 ml of 7.5% sodium carbonate solution was added. The mixture was then incubated at room temperature for 30 min with intermittent shaking. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols and thus produces a blue color upon reaction. The absorbance at 765 nm (due to developed blue color) was determined, using methanol as blank with the help of UV-Visible spectrophotometer [Shimatzu double beam]. The Gallic acid standard curve (absorbance Vs concentration) was prepared to obtain the line of regression (Fig. 1).

Total phenolic content was calculated by putting absorbance of test sample in the line of regression of standard curve of Gallic acid. It is expressed as mg/gm or, µg/mg Gallic acid equivalent (GAE).

Antioxidant assay [Table 3].

DPPH radical scavenging activity [44, 45]

0.1mM DPPH solution (4mg/100ml) and different concentration of test sample in methanol were prepared. 1 ml of test sample was added to 2 ml of DPPH solution and the mixture was incubated at room temperature for 30 min. The absorbance was measured at 517 nm against blank (methanol with DPPH).

% inhibition was calculated as = [(AC 517nm- AS 517 nm/ AC 517 nm) x 100]; AC is the absorbance of the control, and AS is the absorbance in the presence of samples or standards.

Graph of % inhibition and concentration was plotted, using line of regression estimated IC50 [Fig. 5]. The concentration of extract at which 50% inhibition is observed (IC50) is calculated in µg/ml.

Reducing Power Assay [46, 47]

Different concentrations of test sample in methanol (20-100 µg/ml) were prepared. 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (0.5 ml, 1%W/V) was mixed with 0.5 ml of different concentrations of test sample. The reaction mixture was incubated at 50°C for 20 min. After cooling, 1.5 ml of trichloroacetic acid solution (10% W/V) was added to terminate the reaction. At last, 0.5 ml ferric chloride (0.1% W/V) was added and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power because reduced Fe^{2+} into Fe^{3+} increases color intensity of the mixture.

Absorbance versus concentration curve was plotted [Fig. 7].

Hydrogen peroxide Scavenging Assay [48]

Solution of hydrogen peroxide (4mM) in phosphate buffered saline (PBS; pH7.4) was prepared. Different concentration of test sample (20-100 µg/ml) were dissolved in DMSO and added to prepared H_2O_2 solution. Final concentrations of 20 (DMSO only; 0.1 %), 40, 60, 80 and 100 µg/ml were incubated at 20°C for 10 min. The absorbance was measured at 230nm against blank containing 20, 40, 60, 80 and 100 µg//ml samples respectively in PBS, without H_2O_2. Calculations were performed as follows-

% Inhibition = [(AC 230 nm- AS 230 nm/ AC 230 nm) x 100];

AC= control (sample plus phosphate buffer) absorbance, AS= sample absorbance.

A curve for % inhibition vs. concentration was plotted using line of regression and estimated IC_{50} value [Fig. 6].

Statistical analysis

All values are expressed as Mean ± SD (mean and standard deviation) of three replicated experiments. The analysis was performed using Microsoft excel 2007 and SPSS statistical package for WINDOWS (version 16.0; SPSS, Inc., Chicago, IL, USA).

RESULTS

The results of the study are tabulated (Table 1-3) and represented in the form of graphs (Figure 1-7). Preliminary phytoconstituent analysis showed presence of alkaloids, cardiac glycoside, tannin, terpenoids, phenolic and flavonoids in all the extracts. Total phenolic content (Folin Ciocalteu's reagent expressed as gallic acid equivalents, mg/g GAE), Total Flavonoid contents (Aluminium chloride (2%) method expressed as Rutin equivalents RE/g), in vitro antioxidant assays (using; 2, 2-diphenyl-1-picrylhydrazil (DPPH), hydrogen peroxide (H2O2) and reducing power assay (RPA). Comparison of IC50 and % inhibition of the extracts against Ascorbic acid, Rutin and Gallic acid done.The results obtained showed GAE mg/g in the range of order of 537.0 to 240.933 and Rutin Equivalent (RE)/g from 113.6333 to 1049.7.The order of DPPH and H2O2 were found as (IC50 values).

Table 1: Qualitative phytoconstituents analysis

S.NO.	Test	*Carissa carandas*			*Nerium indicum*			*Wrightia tinctoria*		
Tests	Extracts	C1	C2	C3	N1	N2	N3	W1	W2	W3
Glycosides-cardiac/ anthraquinone	Borntrager's test	+++	+++	+++	++	++	+/-	+	+	+
Saponins	Foam test	++	++	++	-	-	++	++	++	++
Oils and fat	Spot test	+	+	+	+++	++	+	+	+	+
Phlobatannins/ Chalcones	HCl test/ spot test	-	-	-	-	-	-	+	-	-
Flavonoids	$AlCl_3$ test/ alkaline reagent test/lead acetate test	+++	+++	+++	+++	+++	+++	+++	+++	+++
Tannins/Phenolic compound	$FeCl_3$ test/ Lead acetate test	+++	+++	+++	+++	+++	+++	+++	+++	+++
Alkaloids	Wagner's test/ Mayer's test/ Hagers/ Dragendorffs	++++	++++	++++	++++	++++	++++	++++	++++	++++
Protein/Amino acids	Ninhydrin/ Xanthoproteic	++	++	++	++	++	++	++	++	++
Steroids	Salkowski's test/ Liebermann buchard	++	+	-	-	-	-	+	-	-
Phytosterols	Sulphuric acid test	+	-	-	+	-	-	+	-	-
Carbohydrates/ sugar	Molish's test/ Benedict's test	+	+	-	+	-	+	+	+	+
Coumarins	Fluorescens test	+	-	-	+	-	-	+	-	

'+' means present, '-' means absent

Table 2: Quantitative Phytoconstituents Analysis: Polyphenol, Flavonoid and Alkaloid contents of various extracts of Carissa, Nerium, and Wrightia (n = 3, X ± SEM). Values expressed in terms of [a]Gallic Acid Equivalent, [b]Rutin Equivalent and [c]Atropine Equivalent respectively. Values assigned with *asterisk are significantly different at $p < 0.05$.

EXTRACTS	[a]TPC-GAE mg/g (y=0.002x + 0.063, R^2-0.995)	[b]TFC- RE mg/1g (y=0.001x + 0.082, R^2= 0.986)	[c]TAC –AE mg/g (y=0.001x + 0.102, R^2 = 0.995)
C1	*357.6667 ± 1.443376	113.6333 ± 0.208167	45.55433 ±0.692074
C2	519.8333 ± 3.329164	*288.1667 ± 2.362908	*38.18753 ±3.153346
C3	278 ± 1.802776	*303.3333 ± 4.129568	30.99667 ± 0.57735
N1	*296.1667 ± 2.929733	1049.7 ± 0.608276	373.33 ± 3.753518
N2	323.7 ± 2.805352	68.76667 ± 3.547299	38.06633 ± 0.3335
N3	*444.8333 ± 3.21455	70.7333 ± 1.101514	61.77667 ± 1.3489
W1	*537 ± 4.769696	97.26 ± 2.205176	142.22 ± 0.696563
W2	*269.4167 ± 2.875906	*163.6 ± 8.170679	*26.77667 ± 2.118875
W3	240.9333 ± 5.688878	*112.3667 ± 1.921805	25.11 ± 1.017988

Values are Mean ± SD of triplicate readings. C1, N1, W1- ethanolic extracts of Carissa, Nerium and Wrightia; N2, C2, W2- Hydroethanolic extracts; C3, N3, W3- Aqueous extracts

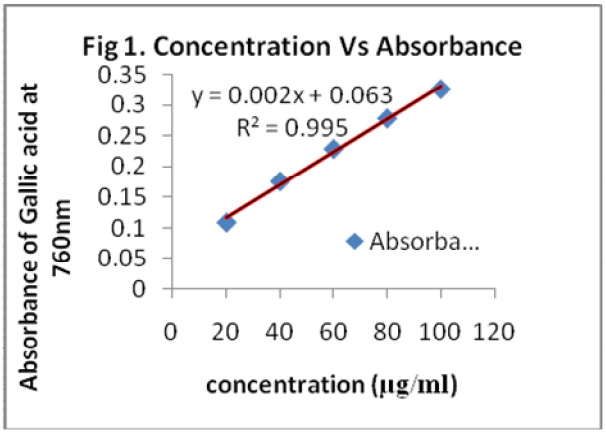

Figure 1.Standard Curve of Gallic acid. All experiments were performed in triplicate. Data are expressed as mean ± SD ($n = 3$, $p < 0.05$) for all concentrations.

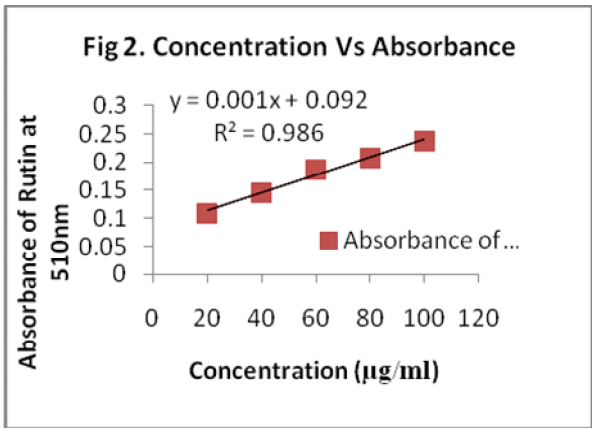

Figure 2.Standard Curve of Rutin. All experiments were performed in triplicate. Data are expressed as mean ± SD ($n = 3$, $p < 0.05$) for all concentrations.

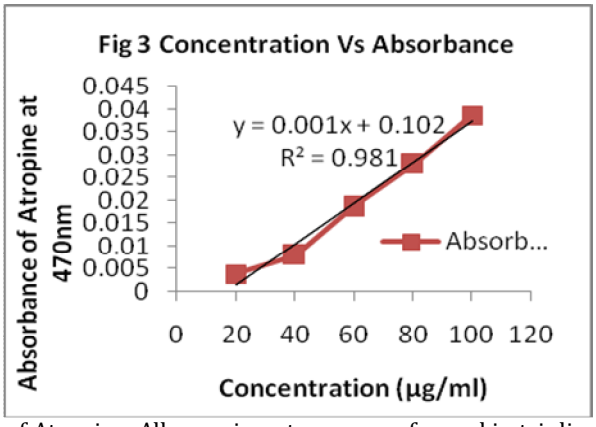

Figure 3: Standard curve of Atropine. All experiments were performed in triplicate. Data are expressed as mean ± SD (n = 3, $p < 0.05$) for all concentrations.

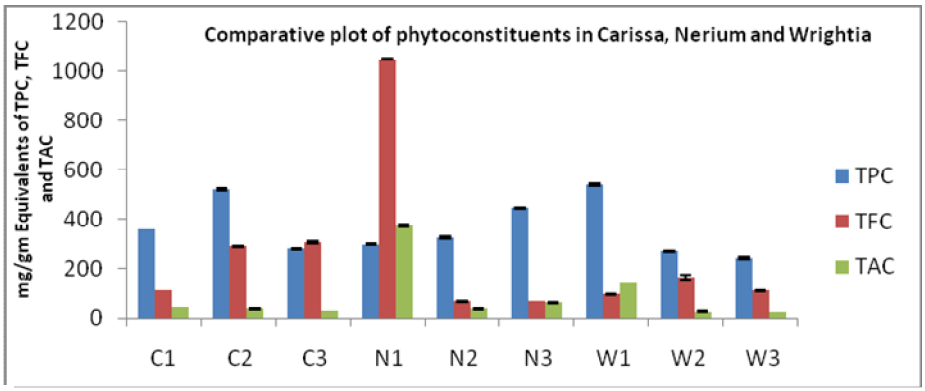

Figure 4. Comparison of Total Phenolic content (TPC), Total Flavonoid content(TFC) and Total Alkaloid content (TAC) of various Ethanolic (C1, N1, W1), Aqueous (C3, N3, W3) and Hydroethanolic (C2, N2, N3) extracts of *Carissa carandas, Nerium indicum, Wrightia tinctoria*. Gallic acid, Rutin and Atropine were used as standard. All experiments were performed in triplicate. Data are expressed as mean ± SD (n = 3, $p < 0.05$) for all readings.

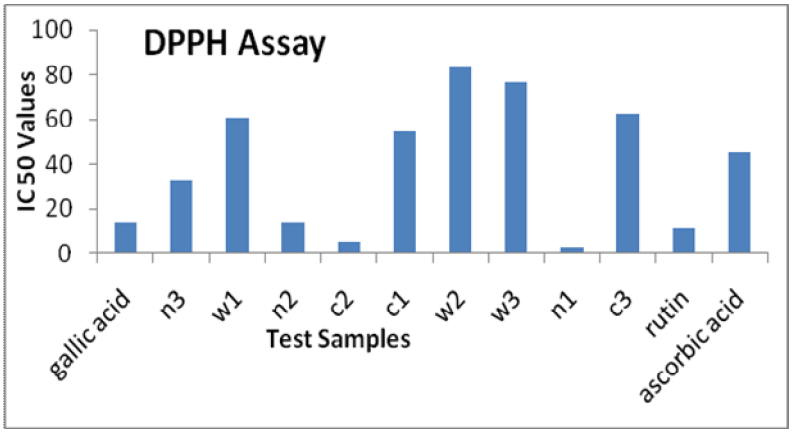

Figure 5. Comparison of IC50 values of various Ethanolic (C1, N1, W1), Aqueous (C3, N3, W3) and Hydroethanolic (C2, N2, N3) extracts of Carissa carandas, Nerium indicum, Wrightia tinctoria, Ascorbic acid (Asc.), Rutin and Gallic acid obtained from DPPH radical scavenging assay. All experiments were performed in triplicate. Data are expressed as mean ± SD (n = 3, $p < 0.05$) for all readings.

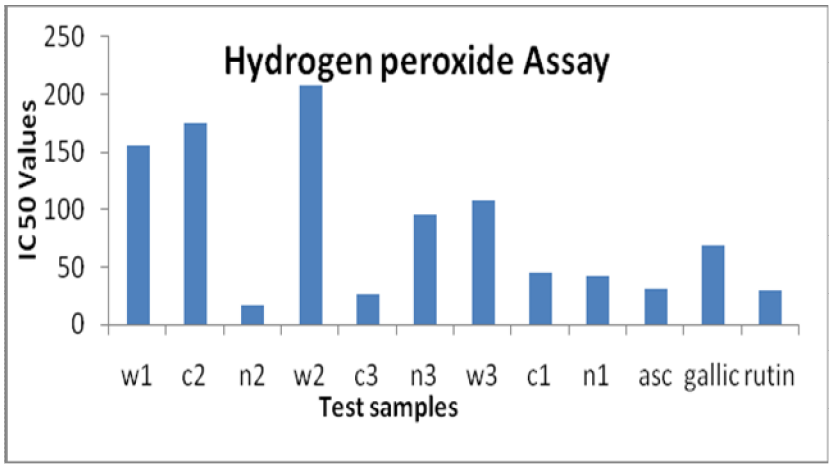

Figure 6. Comparison of IC50 values of various Ethanolic (C1, N1, W1), Aqueous (C3, N3, W3) and Hydroethanolic (C2, N2, N3) extracts of Carissa carandas, Nerium indicum, Wrightia tinctoria, Ascorbic acid (Asc.), Rutin and Gallic acid obtained from Hydrogen peroxide radical scavenging assay. All experiments were performed in triplicate. Data are expressed as mean ± SD (n = 3, $p < 0.05$) for all readings.

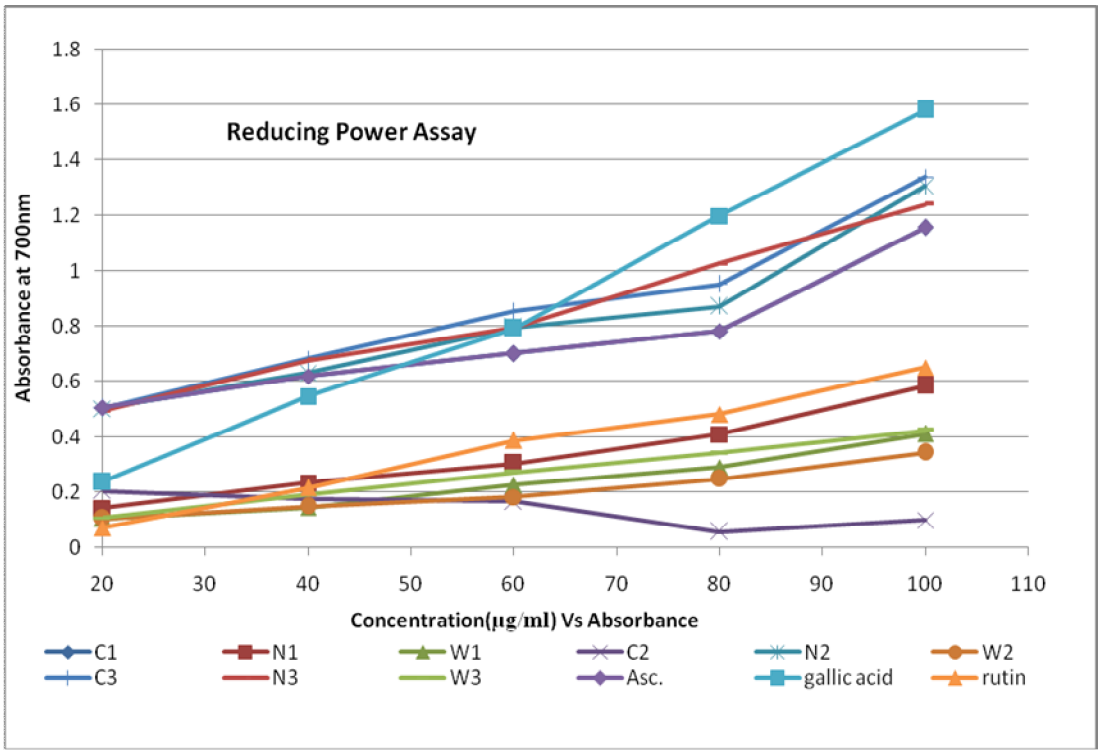

Figure 7. Reducing power of Ethanolic (C1, N1, W1), Aqueous (C3, N3, W3) and Hydroethanolic (C2, N2, N3) extracts of *Carissa carandas, Nerium indicum, Wrightia tinctoria*, Ascorbic acid (Asc.), Rutin and Gallic acid. Values are MEAN±S.D. of triplicate absorbance readings. Each of the extract showed higher activities than control and some showed even higher to the standards (Spectrophotometric color change detection produced by Fe^{+2}–Fe^{+3} transformations). The differences were statistically significant ($P<0.001$ to $P<0.05$). The order of Reducing power of extracts and standard were as: C1>N3>N2>ASC.>W2.

Table 3: Antioxidant Assay (IC 50 values):

Test ---------- Sample	Asc.	C1	C2	C3	N1	N2	N3	W1	W2	W3	Rutin	Gallic acid
DPPH	IC 50	54.077±1.673	4.758±1.6426	62.054±2.3203	2.71056±1.479	13.598±3.8946	32.5606±4.1841	60.5976±9.5191	83.461±1.595	76.41±1.03	11.07 ±9.06	13.43±6.59
H_2O_2 Assay Reducing Power Assay (CONC. & ABS)	IC 50	44.423±5.4591	175.29±7.7022	26.1277±6.5699	42.359±2.8486	15.986±8.2894	95.7173±18.349	155.965±2.5558	207.37±10.67	108.4±8.19	29.42 ±3.804	68.8302 ±2.99
20 µg/ml	0.506 ±0.049	0.143±0.048	0.2207±0.204	0.504 ±0.067	0.229 ±0.112	0.50257±0.15	0.49 ±0.027	0.104 ±0.01	0.1053±0.024	0.106±0.0351	0.071±0.011	0.2376±.039
40 µg/ ml	0.6187±0.027	0.234±0.02	0.3697±0.174	0.6817 ±0.057	0.276 ±0.077	0.630 ±0.105	0.6753 ±0.026	0.145 ±0.027	0.1487±0.037	0.19 ±0.104	0.2157±0.037	0.54627±0.06
60 µg/ml	0.701 ±0.051	0.305±0.02	0.605± 0167	0.85 ±0.051	0.3743 ±0.018	0.7953 ±0.050	0.796 ±0.082	0.2257 ±0.048	0.183 ±0.052	0.273±0.13	0.3877±0.028	0.792±0.1015
80 µg/ml	0.783 ±0.062	0.4113±0.059	0.7697±0.057	0.951±0.088	0.6407 ±0.265	0.873 ±0.047	1.025 ±0.266	0.2907±0.082	0.249 ±0.031	0.342±0.1505	0.483±0.0378	1.1987±0.183
100 µg/ml	1.156±0.397	0.58±0.166	0.8117±0.098	1.3377±0.174	0.935 ±0.552	1.3057±0.435	1.24 ±0.336	0.413 ±0.058	0.3437±0.024	0.424±0.135	0.651±0.048	1.58 ±0.216

Values are Mean ± SD of triplicate readings. C1, N1, W1- ethanolic extracts of Carissa, Nerium and Wrightia; N2, C2, W2- Hydroethanolic extracts; C3,N3,W3- Aqueous extracts and Asc.-ascorbic acid.

DISCUSSION

Plants are continuously being searched for their efficacy to produce certain effects which can improve wellbeing. Single plant has many more species variation depending on regional and climatic differences. Major varieties of Carissa are *C. spinarum, C. edulis, C. lanceolata* and *C. carandas*. Nerium species are *N. oleander/odorum* white/ pink variety and Wrightia come in *W.tomentosa and W. tinctoria*. These plant species are searched for Novel antioxidants by many authors.

Ahmed H. El-desoky *et al*., [49] isolated Naringin from methanolic extract of leaves and superoxide and DPPH radical scavenging activity. NG showed significant antioxidant activity as it scavenged the superoxide radical (EC_{90}, 10.95 µM) and DPPH radical (EC_{50}, 11.2 µM).

Normaizatul Afizah, *et al.,* [50] antioxidant properties, total polyphenol contents, H2O2 reducing capacity, cytotoxic potentials, reducing power and DNA damage inhibition potential of an ethanolic and n-hexane extracts of *Carissa carandas* fruits were determined.

Verma *et al*., [51] confirmed dose-dependent free radical scavenging effects *Carissa carandas* leaves extract in methanol using DPPH (73.1 µg/ml), H_2O_2(84.03 µg/ml) and reducing power activity and total antioxidant activity in-vitro models . Also, it protected pBR322 plasmid DNA assessed using DNA damage inhibition assay. Methanolic extract of *C. carandas* leaves showed high content of phenolic compounds (84.0 mgGAE/g dry wt), estimated using Folin–Ciocalteau assay [51].

Saikia and Handique [52] ethanolic extract of the fruit, leaf and seed of *C. carandas* observed for antioxidant activity individually using DPPH and H2O2 radicals model. The order of antioxidant potential the extracts were: Fruit > Leaf > Seed [52].

Karunakar H. *et al*. [53] evaluated membrane lipid peroxidation and antioxidant activity of ethanolic extract of *Carissa carandas* roots. Results showed higher effects on increasing concentration as (IC50 values); ABTS, DPPH, super oxide, nitric oxide, erythrocyte haemolysis; 324.93, 185.08, 117.66, 242.69 and 70.82 µg/ml respectively. Membrane lipid peroxidation induced thiobarbituric acid reactive substances (TBARS) and FeSO4/ascorbate on rat liver homogenate showed effects in concentration dependent manner [53].

Khatun M *et al*., [54] *Carissa carandas* leaves (MELC) extracted in methanol and The DPPH and ABTS assays performed. The antioxidant activity of MELC with IC50 10.5±1.2 and 1.75±0.3µg/ml that was comparable to L-ascorbic acid [54].

Aniruddha Sarma *et al* [55] the antioxidant activity was evaluated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals and the IC50 value of methanolic extract of *C. carandas* fruits was found to be 27.45±0.43µg/ml. Among the nutraceutical properties carbohydrate (19.32 ± 0.69 mg/100g), protein (0.185 ± 0.011µg/g), crude fibre (15.64 % ±0.64), total phenolic content (188.75 ± 1.42 µgGAE/g) and ascorbic acid (62.93 ± 0.35 mg/100g) were recorded [55].

Yeşilada *et al.,* [56] evaluated Antioxidant properties of *Nerium indicum Linn*. Flower extract using DPPH, FRAP and CUPRAC assays. Total flavonoids and Phenolic contents were determined using the AlCl3 and FC reagents, respectively. Ethylacetate and ethanol extract showed highest antioxidant capacity. Maximum phenolic and flavonoid contents were present in ethanolic extract.

Azeez *et al.,* [57] revealed the nutritive and antioxidant potential (DPPH and FRAP assay) of *Carissa carandas* fruit without seeds. The fruit pulp came out as good source of phenols, flavonols and anthocyanins, the amounts of phenols and flavonoids. Presence of phenolic acids, revealed high concentrations of vanillic, protocatechuic, t-cinnamic, ferulic, chlorogenic, 2,4-dihydroxy benzoic, syringic and salicylic acids; flavonoids rutin, myricetin and umbelliferone; and glucosides of the anthocyanins cyanidin and pelargonidin. Susanta Kumar Rout *et al.,* [58] evaluated antioxidant activity of different fractions of methanolic extract of *Nerium oleander Linn.* Leaves.

Singhal KG and Gupta GD [59] evaluated In vitro antioxidant activity of methanolic extract of flowers of *Nerium oleander* (MENO-F) using reducing power, lipid peroxidation, DPPH, ABTS, superoxide anion, hydroxyl radicals and metal chelation.

Priyanka Dey (2012) screened antioxidant effect of individual parts of *N. indicum* (white flowered variety) using in-vitro models total antioxidant, DPPH radical and singlet oxygen scavenging potential [60] . Sreena *et al*. [61] methanolic, ethanolic and ethyl acetate extracts of in vitro radical scavenging efficacy of different organic extracts of *Nerium indicum* leaves [61].

Fatima N., *et al*. [62] performed antioxidant activity using (DPPH) radical scavenging activity and (ABTS) radical cation decolorization assay on *Wrightia tinctoria* bark methanolic extract (WTBM). The total phenolic content of was found to be 30.3 GAE mg/g dry weight of bark extract while IC50 value for DPPH and ABTS radical scavenging activity was 72.2 ± 2.8 µg/ml and 45.16 ± 1.95 µg/ml, respectively. HPLC analysis showed the presence of gallic acid, rutin, and quercetin in WTBM [62] . Jesy and Jose [63] investigated on cytotoxic, antioxidant, antimicrobial and volatile profile of Wrightia tinctoria (Roxb.) R. Br. Flowers [63]. N Edaydulla *et al.,* [64] conducted study on ethanolic flower extract of W.tinctoria and found total phenols, flavonoids, carotenoids to be 55.29±0.45 mg GAE, 370.53±1.213 mg QE and 1.825±0.321 mg/g respectively. DPPH Assay (IC50) of the flower extract was 43.16µg/mL.

Antioxidant activities of several medicinal plants produced promising results. Antioxidant plants are helpful as neutraceuticals, adjuvant therapy, supplements, wine making, and tanneries and as meat freshners etc. Antioxidant activity of plants were assessed using multiple in-vitro and *in vivo* models and the most popular model among the scientific community were DPPH, FRAP, ABTS, H2O2 and Reducing power assay etc. The phytoconstituents like Flavonoids, anthocyanins, Phenolic compounds, Alkaloids, Terpenoids, Tannins and Saponins attributed for the antioxidant effects.

Various studies reported that the role played by phytoconstituents on meristematic cell is similar to that played on human cells. Secondary metabolites are generated as a result of homeostasis in plants or they could the by-products of various metabolic/ biochemical processes within the cell including primary metabolites. Oleandrin, odoroside, indirubin, Naringin, wrightial, carrindone, ouabain, ellipticine, vinca alkloids, carrisol etc are very important leads from this family.

CONCLUSION

Carissa carandas, Nerium indicum and *Wrightia Tinctoria* belonging to Apocynaceae family could are having moderate to high antioxidant activity. It is always better to avoid derooting of plants. Aerial parts of any plant could give maximum phytoconstituents in the extract.

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CONFLICT OF INTEREST

none

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