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Antioxidant activity of *Nothopegia racemosa* (Dalz.) ramam

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Abstract
Phytochemicals are naturally occurring bioactive compounds produced during normal metabolic activities. These are helpful for mankind to overcome several health risks and improve body to anti allergic, antimicrobial, anti-inflammatory action. Worldwide plants have been the source of traditional medicine. In recent time there is growing interest for food additives and dietary supplements of natural origin to maintain good health. Preliminary phytochemical screening investigated using leaf of *Nothopegia racemosa* in different solvents like Hexane, chloroform, Petroleum ether, Hydro alcohol (50% alcohol) and Aqueous reveals the presence of bioactive compounds such as alkaloids, Phenols, Flavonoids, Tannins, saponins, steroids, carbohydrates and Protein**.** Since the experimental plant has potential phytoconstituents, free radical scavenging activity by 1,1-Diphenyl-2-picryl hydroxyl(DPPH) quenching assay is conducted in Hexane, chloroform, Petroleum ether, Hydro alcohol and Aqueous leaf extracts. Results obtained shows IC50 value of 380.7 µg/ml in Hydro alcohol (50% alcohol) and 418.8 µg/ml in Aqueous extract indicating potential antioxidant activity of crude extract.

Keywords: *Nothopegia racemosa*, antioxidant activity, DPPH, free radical

Introduction
Plants synthesize bioactive compounds to perform normal physiological functions or to protect themselves from pathogens or to respond towards environmental stress. Plants with such medicinal properties have long been used by rural communities in traditional food and in folk medicine. But many of these are not commercialised due to lack of knowledge on their nutraceutical composition and popularity. Two third of the world's flora augment with medicinal properties along with antioxidant activity. (Krishnaiah *et al.,* 2011) [14] Antioxidants are substances formed in the body. These slow down or prevent cell damage caused by free radicals produced in the body as a reaction to pressure. Antioxidants may be endogenous (produced in the body) or exogenous (taken from outside), the sources may be natural or artificial. Free radicals, also called as reactive oxygen species are waste substances produced by inflammation or when a body reacts to environmental factors like UV rays, pollution**.** Its production increases under stress. If the body do not eliminate free radicals by processes it causes oxidative stress resulting in pathogenesis. Major group of phytochemicals contribute to antioxidant capacity and possess the ability to scavenge both active oxygen species and electrophones. (Nataraj *et al.,* 2010) [18] Antioxidants have the ability to take up the free radicals, lower the burden and make it stable (Aruoma, *et al*, i2003) [4]. Antioxidants are group of substances which, when present at low concentrations, in relation to oxidiziable substrates, being oxidized them, inhibit or delay oxidative processes. (Rhee *et al.,* 2009) [20]. Free radical oxidative stress has implications in the pathogenesis of a variety of human diseases such as atherosclerosis, diabetes mellitus, hypertension, inflammation, cancer and AIDS (Halliwell 1989) [12]. Infectious, neurodegenerative and auto immune diseases are due to cell damage caused by oxidative stress. (Ulewicz 2019) **[24]** In human insufficient diet leads to oxidative stress causing damage of cell, DNA causing cancer, Diabetes etc**.**. (Deepak shashank2020) [9] *Invitro* studies have proved the production of antioxidant activity showing Phenolic acids, Tannins, Vitamin C, and Vitamin E under oxidative stress by functioning of reducing agents, free radical scavengers and metal chelaters. **(**Deepak *et al.,* 2015) [8] In recent days investigation of natural antioxidants from plant source is increasing (Ghani *et al.,* 2019) [11] Production of sustainable bio products from naturally derived antioxidants and their use in diet helps to maintain health**.** (Asgarpanah 2014) [5]

Most of the plant parts like leaves fruits, seeds are used by rural communities in traditional food or in folk medicine. But these are not commercialised due to lack of knowledge on their nutraceutical composition and popularity. Preliminary qualitative analysis carried out using leaf of *Nothopegia racemosa* in different solvents like Hexane, chloroform, Petroleum ether, Hydro alcohol and Aqueous reveals that it is treasure of bioactive compounds such as alkaloids, Phenols, Flavonoids, Tannins, Terpenoids and steroids, carbohydrates Protein etc. In this context present investigation of antioxidant activity evaluation in *N. racemosa* is undertaken by DPPH assay. The DPPH scavenging assay is a simple, most frequently used for *in vitro* antioxidant activity evaluation for the any compound. (Alam *et al.,* 2013) [2].

Material and Methods
Plant material collection
Plant sample of *N. racemosa* was collected on 16th June 2019 from their natural habitat Western Ghats region in Thattapura forest, Shimoga District, Karnataka, India. The

plant was identified by taxonomist and the voucher specimen is deposited at Department of Studies and Research in Botany, Tumkur University Tumakuru. The identification number is TUBNR-2018 Leaves were separated from twigs, washed to get rid of dust. It is shade dried for 21 days to make it free from moisture content, mechanically powdered, sieved and stored in air tight containers.

Preparation of Extract

The powdered leaf sample was extracted with different solvents of varying polarity. 20 gms of sample powder was Weighed, dissolved in 100 ml of different solvents Chloroform, Petroleum ether, Hydro alcohol (50% alcohol), Hexane aqueous in 500 ml beaker and covered with aluminium foil. Then the beaker was kept on hot water bath at 50^0C for 4 hrs. After incubation the extract was filtered with whatmann

filter paper, the filtrate was collected in 50 ml beaker. The residue was discarded and filtrate was kept at 50^0C for few hours until extract is completely dried. The semisolid form of samples are weighed, the weight was recorded and later taken for further analyses.

Reagents used

DPPH (EEC No. 217-591-8, Sigma, USA) stored at less than 0°C, Methanol HPLC grade (Ranbaxy Chemicals) and Quercetin (1mg/ml) Inhibitor (reference standard).

Preparation of working solutions.

1mg DPPH was dissolved in 6 ml HPLC grade methanol. 1mg of Quercetin was dissolved in 1ml methanol.

Procedure

DPPH solution of 80µl, test solution of different Concentrations were prepared to sufficient quantity of 240 µl with HPLC grade methanol. Reference standard are tested for various concentrations of 0, 0.3125 0.625, 1.25, 2.5, 5 and 10 µg/ml. Test samples of different concentrations like 10, 20, 40, 80, 160, 320, 640 µg/ml are tested. Then the reaction mixture was mixed and incubated for 15 minutes at 25°C. Using semi-auto analyzer the absorbance was read and recorded at 517 nm. Absorbance for Control reaction that contains all reagents without the test compound is carried out. The concentration of substance needed to inhibit biological process or a function by half is referred as half maximal Inhibitory concentration (IC50).

IC50 value was not calculated for the Compounds which shows inhibition less than 50%, for various concentrations of different test sample and standard the % inhibition was recorded and calculated using formula

$$\text{Inhibition} = \frac{(\text{OD of Control} - \text{OD of Sample})}{\text{OD of Control}} \times 100$$

The relative activity of the test sample was determined by comparing the IC50 value of sample with standard and IC 50 is calculated using Graph Prism software version 5.0 by nonlinear regression.

Result

Phytochemical screening *of N.racemosa* leaf in chloroform, Hexane, Hydro alcohol, Petroleum ether and Aqueous solvent extract reveals that it is a treasure of bioactive compounds such as alkaloids, Phenols, Flavonoids, Tannin and steroids, carbohydrates and proteins.

Radical scavenging is carried out by DPPH method. Quercetin is used as reference standard. The plot of % inhibition against concentration, from data at table 1, is showed by figure 1.The concentration of extract by different solvents used to determine IC50 values ie. The amount of extract needed to scavenge DPPH radicals 50% from data is shown in the plot of % inhibition against concentration in Figure 2 and 3.

Table 1: Calculating percentage growth inhibition of standard

Sample Name	Concentration (µg/ml)	Absorbance 517nm	% Inhibition	IC50 (µg/ml)
*Standard (*Quercetin)	0	0.763	0.0	**1.736**
	0.3125	0.687	10.0	
	0.625	0.635	16.8	
	1.25	0.489	35.9	
	2.5	0.348	54.4	
	5	0.291	61.9	
	10	0.161	78.9	

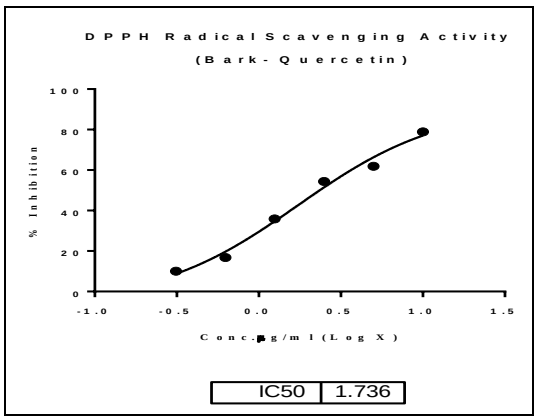


Fig 1: DPPH Radical scavenging –Quercetin

Table 2: Crude Leaf sample of *N.racemosa* showing Absorbance at 570 nm, % Inhibition and IC50 (µg/ml) in different Solvents

Crude sample	Concentration (µg/ml)	Absorbance 517nm	% Inhibition	IC50 (µg/ml)
Chloroform	0	0.763	0.0	Activity less
	10	0.725	5.0	
	20	0.652	14.5	
	40	0.628	17.7	
	80	0.573	24.9	
	160	0.502	34.2	
	320	0.453	40.6	
	640	0.392	48.6	
Hexane	10	0.736	3.5	Activity less
	20	0.685	10.2	
	40	0.622	18.5	
	80	0.569	25.4	
	160	0.511	33.0	
	320	0.463	39.3	
	640	0.401	47.4	
Hydro Alcohol	10	0.702	8.0	380.7
	20	0.658	13.8	
	40	0.593	22.3	
	80	0.513	32.8	
	160	0.456	40.2	
	320	0.396	48.1	
	640	0.184	75.9	
Petrolium Ether	10	0.724	5.1	Activity less
	20	0.665	12.8	
	40	0.622	18.5	
	80	0.555	27.3	
	160	0.442	42.1	
	320	0.411	46.1	
	640	0.391	48.8	
Water	10	0.711	6.8	418.8
	20	0.661	13.4	
	40	0.594	22.1	
	80	0.562	26.3	
	160	0.461	39.6	
	320	0.444	41.8	
	640	0.193	74.7	

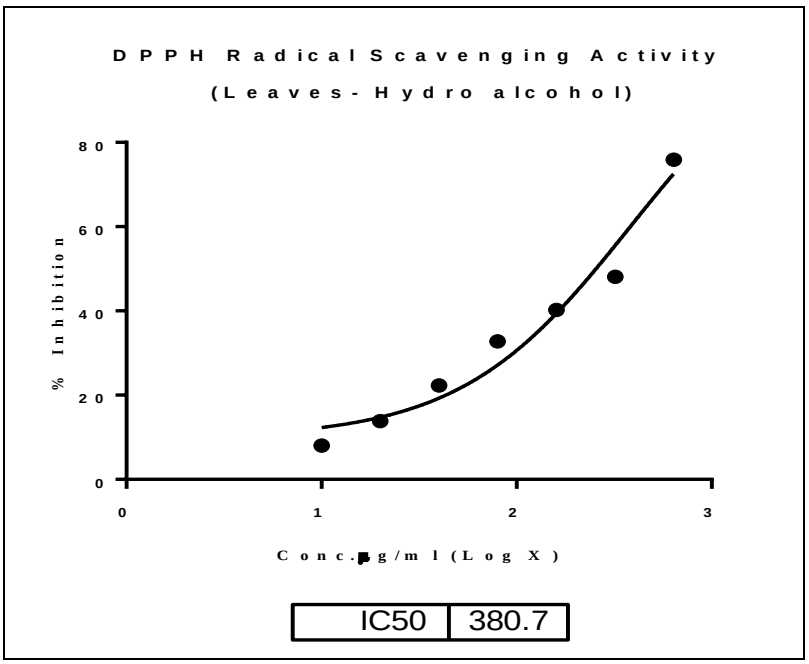


Fig 2: DPPH Radical Scavenging of *N.racemosa* in Hydro alcohol

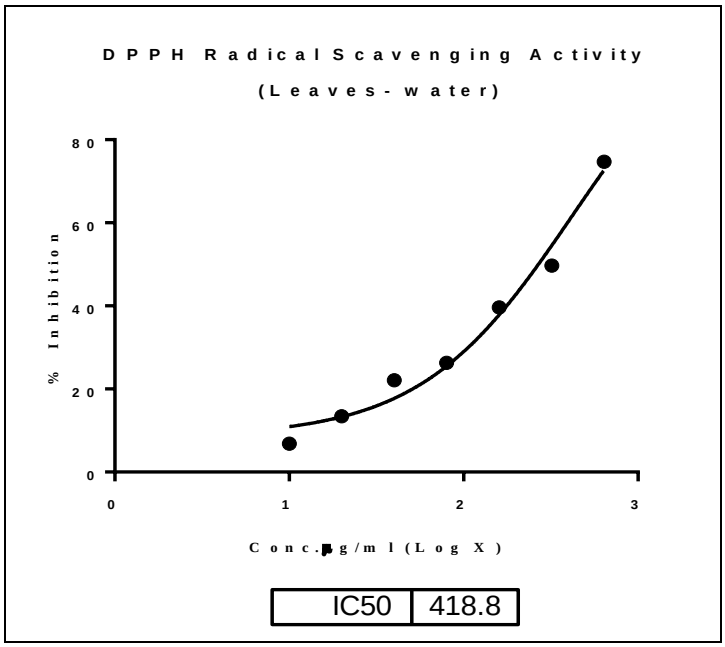
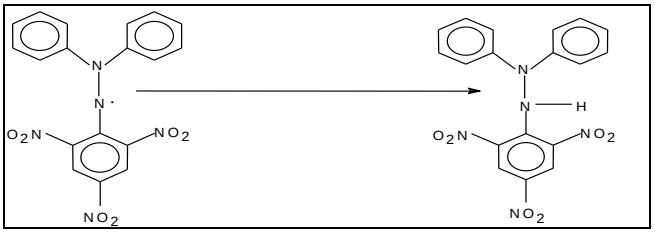


Fig 3: DPPH Radicle Scavenging *N.racemosa* for Aqueous

Discussion

Phytonutrients like Alkaloids, Flavonoid, Steroid, Tannins present in leaf extract of Anacardium occidentale are responsible for antioxidant, immunomodulator, antiulcer activity and has positive effects on human health. (Dharamveer *et al.,* 2013) [10] Oxidative process produces free radicals which contain unpaired electrons which can damage DNA and attack Lipids and Proteins Anacardium plants contains remarkable number of secondary metabolites in leaves and fruits which Posses therapeutic properties of which prominent use as Antioxidants, antimicrobial and Anticancerous (Bahara *et al.,*2020) [6]. Phytochemical screened for leaf in five different solvents namely Hydro alcohol, Hexane, Petroleum ether, water and Chloroform Shows presence of Alkaloids, Phenols, terpenoids and Carbohydrates in all solvents. Flavonoids in all solvents except Hexane. Since last decades search for natural dietary antioxidants by DPPH method is increasing. DPPH [1, 1-diphenyl-2-picryl hydrazyl] is a stable free radical with purple colour, when mixed with a substance that donates hydrogen atom, it loses its purple colour as antioxidant reduces it to 1, 1-diphenyl-2-picryl hydrazine and becomes colourless or pale yellow colour compound which is measured at an absorbance 517 nm**.** IC 50 value is the parameter considered to analyse the potentiality of an antioxidant of compound in test sample which can scavenge free radicals by 50% indicating close correlation between concentration and % inhibition. (Marjoni *et al.,* 2017) [15]



1, 1-diphenyl-2-picryl hydrazy l 1, 1-diphenyl-2-picryl hydrazine (Purple coloured) (Colourless)

Fig 4: Conversion of 1, 1-diphenyl-2-picryl hydrazyl to 1, 1-diphenyl-2-picryl hydrazine

DPPH is a free radical compound which has scavenging ability for antioxidants samples and shows good absorbance at 517 nm (Juan *et al.,* 2010)[13] The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure antioxidant activity.(Mounir *et al.*,2020) [17]

In the present study Table 2 shows different concentration test sample, % of inhibition as antioxidant activity and IC 50 of Leaf in crude extract in various solvents. DPPH radical-scavenging activity in Water extract is greater with IC50 value of 418.8 μg/ml than the Hydro alcohol with IC50 value of 380.7 μg/ml. Radical-scavenging activity increases with increase of concentration of extract. All solvent extracts and fractions of leaf showed lower absorbance with higher concentration. Percentage of antioxidant activity in water extract is better than the Hydro alcohol. The Chloroform, Hexane, Petroleum ether shows <50% inhibition. And exhibited varying degrees of scavenging capacities. Quercetin is used as reference standard with IC50 value of 1.736 µg/ml.

Similar to present study with Quercetin used as reference standard**,** *Invitro* estimation of potentiality of quercetin for antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) shows high reducing capacity with IC50 values 36.15±0.30 mg/ml when compared to the standards ascorbic acid IC50 value 31.45±1.3120 mg/ml. (Srimathi priyanga. *et al.,* 2017) [22] Stem bark of *Spondias purpurea* L in methanolic extract shows better Free radical scavenging activity with8.3±1.5151µg/ml. Taiwo and Tomayo (2018) [23]. Antioxidant activity in methanolic leaf extracts of Cotinus *coggygria* (Ali *et al.,* 2015) [3].Similar to *N.racemosa* by DPPH method exhibits potent antioxidant activity with % of inhibition of 95.69 ± 0.17 at 517 nm absorbance. In water extract *N.racemosa* lea*f* shows high antioxidant activity % inhibition at 80, 160, 320, 540 µg/ml was 26.3%, 39.6 and 41.8 and 74.7% respectively when compared to antioxidant activity of leaf in water extract of *Coffee Arabica* at % inhibition at 80µg/ml was 50.9% and at 100µg/ml was 58.3%. (Mauizatul et *al.,* 2016) [16] Methanol leaf extract of Garcinia morella shows scavenging activity of 30.81% at 100 µg/ml, and IC 50 value of 225.70 µg/ml

similar to 32.8% at 80 µg/ml and IC 50 value of 380.7 µg/ml in Hydro alcohol leaf extract of *N.racemosa* (Vishak *et al.*,2017) [25] *Withania somnifera* plant extract free radical scavenging by DPPH assay in Ethanol + diethyl ether +N-Butanol, Water, Ethanol, Methanol, Ethyl acetate, Chloroform extract exhibited % of inhibition of 1mg/ml of polar flavonoid 83.07%,, Saponin, 65.80%, non-polar flavonoid 67.87%, terpenoid 70.29%, tannin exhibited70.63%. Antioxidant activity of *N.racemosa* leaf supports it as it is also rich in Flavonoid, Phenol, Saponin, Tanin and Terpenoid. (Abdul Qaiyum *et al.,* 2013) [1]
Methanol extracts of *Rhus coriaria* (Anacardiaceae) shows great antioxidant activity against free radicals and lipid per oxidation *in vitro with* IC50 value 1200 µg/mL in Fe+2-ascorbate system, superoxide-scavenging activity in txanthinex anthine oxidase method 282.92 µg/ml and hydroxyl radical scavenging activity in deoxyribose decomposition method 3850 µg/ml (Candan and Sokmen 2004) [7] While *N.racemosa* shows IC50 value in Hydro Alcohol 380.7 and water Hydro Alcohol 418.8µg/ml by DPPH assay. *Mamecylon* species in methanol extract of leaf for standard Quercetin shows IC50 +1.75 .µg/ml and 32 species shows IC50 value of 15.30 µg/ml in *Mamecylon heyneanum* to 232.47 µg/ml in *M. terminale* Dalzell. Similar to this *N.racemosa* shows IC Value of 1.736 µg/ml for reference standard quercetin and IC value of *Hydro Alcohol* 380.7 and water *Hydro Alcohol* 418.8µg/ml.(Sivu and Pradeep 2013) [21] Linear correlation between phenolic content of plant extracts and antioxidant activity is proved by investigations. (F. Pensec *et al.,* 2016) [19]

Conclusion
Immunity can be enhanced by adding natural antioxidants in diet. These neutralise effect of free radicals in our body and maintain human health. The *invitro* studies of leaf extracts in Hydro alcohol, Hexane, Petroleum ether, water and Chloroform shows decrease in absorption of DPPH radical scavenge values with increasing concentration along with increase in inhibition percentage. It proves significant, strong antioxidant potentiality.

Conflict of Interest
The author declared that there is no conflict of interests regarding the publication of this paper.

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