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Vetting and Comparative Analysis of Antioxidants and Phytochemicals from Methanolic, Aqueous and a Popular Commercial Fruit Juice (Noni) *Morinda Citrifolia L.*

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ABSTRACT

Noni (*Morinda citrifolia*) *L* has been extensively used in Folk medicine by Polynesians for over 2000 years. It has been reported to have broad therapeutic effects, including anticancer activity, in both medicinal practice and laboratory animal models**.**This study was carried out to evaluate the phytochemical constituents and antioxidant activity of a popular commercial brand of Noni fruit juice and aqueous and methanolic fruit extract of *Morinda citrifolia L* prepared from fruits. The extracts contain phenols, flavanoids, sugars and tannins and showed significant antioxidant activity against DPPH, Nitric oxide, Superoxide anion and Hydrogen peroxide**.** Methanolic extract contains more plethora of bioactive compounds in comparison to that of aqueous and commercially available fruit extract.

Keywords: Antioxidant, anticancer, oxidative stress, Reactive Oxygen Species, Radical Scavenging Activity,

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INTRODUCTION

India is known for its rich diversity of medicinal plants and from ancient times these plants have been utilized as therapeutic agents. Today's research is mainly focused on medicinal plants because the bioactive compounds and medicinal power mainly depends on phytochemical constituents that have great pharmacological significance [1]. Plants synthesize potent biochemicals which constitute the components of phytomedicine since times immemorial [2]. Medicinal plants typically contain several different pharmacologically active compounds that may act individually, additively or in synergy to improve health [3]. These phytochemical constituents, natural bioactive compounds, nutrients and fibers present in medicinal plants, fruits and vegetables defend us from various ailments [4]. *Morinda citrifolia* fruit is rich in phytochemicals such as flavinoids, alkaloids, tannins, sugars and phenols.

Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress. Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process [5]. This concept is supported by increasing evidence that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants oppose this and lower risk of disease [4]. Antioxidants when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevent oxidation of that substance [6].

Apart from their role of health benefactors, antioxidants are added in foods to prevent or delay oxidation of food, initiated by free radicals formed during their exposure to environmental factors such as air, light and temperature [3]. At present most of the antioxidants are manufactured synthetically. They belong to the class of synthetic antioxidants. The main disadvantage with the synthetic antioxidants is the side effects when taken in vivo [4].

Morinda citrifolia L. fruit contains a number of antioxidants such as beta-carotene, ascorbic acid, terpenoids, alkaloids, beta-sitosterol, carotene, polyphenols such as flavonoids, flavone glycosides, rutin etc. [4]. Easily cultivable *Morinda citrifolia* L. with its wide range of antioxidants can be a major source of natural or phytochemical antioxidants.

Knowledge of the phytochemical profile of processed *Morinda citrifolia* (noni) fruit is also important in understanding potential bioactivities, as well as in identifying the compounds responsible for beneficial effects already demonstrated in human clinical trials [7]. A few publications have provided limited phytochemical and antioxidant information on the composition of *Morinda citrifolia L* (noni) juice.

This study was carried out to evaluate the phytochemical and antioxidant content of a popular commercial Noni product and compare it to aqueous and soxhlet methanolic extract of *Morinda citrifolia L* prepared from fruits.

MATERIALS AND METHODS

Commercial Noni juice available in 10mL solution was purchased from BSY Marketing India Pvt. Ltd. Noni Juice and was diluted in doubled distilled water.

Plant Material

The fruits of *Morinda citrifolia* were purchased from Abirami Botanical Tamil Nadu India. Specimen was authenticated from St. Xaviers College, Department of Botany, Mumbai as *Morinda citrifolia L* belonging to family Rubiacese with Blatter Herbarium specimen number 108.The fruits were air dried for 2 days and ground to powder.

Preparation of extracts

The active principles from the powdered fruits of *Morinda citrifolia L* was extracted with absolute methanol using a Soxhlet extractor exhaustively for 20-24 hours. The extracts were concentrated to dryness

under reduced pressure and controlled temperature (40-50°C). The dried extract (yield) obtained were used in this study [4].

The aqueous extract was prepared by cold maceration of 250 gms of the shade-dried fruits powder in 500 ml of distilled water allowed to stand overnight, and boiled for 5-10 minutes till the volume was reduced to half its original volume. The solution was then cooled, filtered, concentrated, dried in vacuum (yield 36 g) and the residue stored in a refrigerator at 2-8°C for subsequent use [7].

Estimation of Phytochemicals and Antioxidants

The extracts of the fruits of *Morinda citrifolia* were analyzed for the presence of various phytoconstituents using standard protocols.

Estimation of total phenol content (TPC)

The total phenol content was determined by Folin- Ciocalteu method. [8]0.5 ml of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5N) were mixed and incubated at room temperature for 15 min. 2.5 ml saturated sodium carbonate was added and after 30 min absorbance measured at 760 nm.[9] The total phenol content was expressed in terms of gallic acid equivalent (µg/ml).

Estimation of total flavonoids (TF)

The total flavonoid content was determined by aluminum chloride method. [8]The reaction mixture comprising of extract, aluminum chloride (1.2%) and potassium acetate (120 mM) was incubated at room temperature for 30 min and absorbance was measured at 415 nm. The total flavonoid content was expressed in terms of quercetin equivalent (µg/ml) [10].

Estimation of sugars.

The extract was done by DNSA method [1]. 1 ml of the extract was added to 1 ml DNSA. The contents were mixed and boiled for 5 min. 2 ml of distilled water was added to the mixture and absorbance was measured at 525 nm. Sugar content was expressed in terms of maltose equivalent (µg/ml).

Estimation of tannins.

The tannin content was determined by Folin-Ciocalteu reagent method. 0.5 ml of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5N) were mixed and incubated at room temperature for 15 min. 2.5 ml saturated sodium carbonate was added and further incubated for 30 min at room temperature and absorbance measured at 760 nm. The tannin content was expressed in terms of tannic acid equivalent (µg/ml) [11].

Estimation of chlorophyll.

0.2 gms /10ml of sample was analysed. The contents were transferred to a centrifuge tube and the total volume was made up to 10ml with distilled water. 0.5 ml from the tube was transferred to a tube containing 4.5ml of 80% acetone. The contents were centrifuged at 4000 rpm for 15 min. The absorbance of the supernatant was measured at the following wavelengths-645,663,490,638 nm [12] and the content of chlorophyll was calculated [13].

Evaluation of antioxidant activity

α, α-diphenyl- β-picryl-hydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picrylhydrazyl or 1, 1-diphenyl-2-picryl-hydrazyl by the method of McCune and Johns. [14] The reaction mixture consisted of DPPH in methanol (0.3 mM) and extract. After incubation for 10 min in dark, the absorbance was measured at 517 nm [11]. DPPH scavenging activity was expressed in terms ascorbic acid equivalent (ug/ml).

Nitric oxide (NO) radical scavenging assay

Sodium nitroprusside (3.0 ml) in phosphate buffer (10 mM) was added to extract and incubated at 25°C for 60 min. To 5.0 ml of the incubated sample, 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H_3PO_3) was added and absorbance of the chromophore formed was measured at 540 nm. NO radical scavenging activity was expressed in terms of ascorbic acid equivalent (µg/ml) [9, 11].

Ferric reducing antioxidant power (FRAP) assay

Extract(0.2ml) was added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM $FeCl_3$. $6H_2O$ solution) and the reaction mixture was incubated at 37°C for 30 min and the increase in absorbance at 593 nm was measured [9]. The antioxidant capacity based on the ability to reduce ferric ions of sample was expressed in terms of ascorbic acid equivalent (µg/ml).

Estimation of reducing power (RP)

The reducing power was determined by the method of Athukorala [1]. 1.0 ml extract was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50°C for 20 min. 2.5 ml of trichloroacetic acid (600 mM) was added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution was diluted with distilled water and 0.5 ml of $FeCl_3$ (6 mM) and absorbance was measured at 700 nm. RP was expressed in terms of standard equivalent (µg/ml) [9].

Superoxide anion (SO) radical scavenging assay

The superoxide anion scavenging activity was measured as described by Robak and Gryglewski [21]. The superoxide anion radicals are generated in 3.0 ml of Tris-HCl buffer, containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH solution (0.936 mM), 1.0 ml extract and 0.5 ml Tris-HCl buffer (16 mM, pH 8). The reaction was started by adding 0.5 ml PMS solution to the mixture, incubated at 25°C for 5 min and then the absorbance was measured at 560 nm. SO anion scavenging activity was expressed in terms of ascorbic acid equivalent (µg/ml) [17].

Hydrogen peroxide (H2O2) radical scavenging assay

The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of Ruch [5]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4) and 2 ml of the solution was added to 1 ml extract. The absorbance at 230 nm was determined after 10 mins. H_2O_2 radical scavenging activity was expressed in terms of ascorbic acid equivalent (µg/ml) [9].

Total antioxidant

The antioxidant activity was determined according to the method [15] 3ml of reagent solution (0.6M sulphuric acid 28mM sodium phosphate and 4mM ammonium molybdate).

Reaction mixture was incubated at 95^{o}C for 90 mins in a water bath. Absorbance of all the sample mixture was measured at 695nm. Total antioxidant activity is expressed as the number of equivalence of ascorbic acid .A calibration curve of ascorbic acid was prepared and the total antioxidant activity was standardized against ascorbic acid and expressed as mg ascorbic equivalent per gram of sample dry weight [1,9].

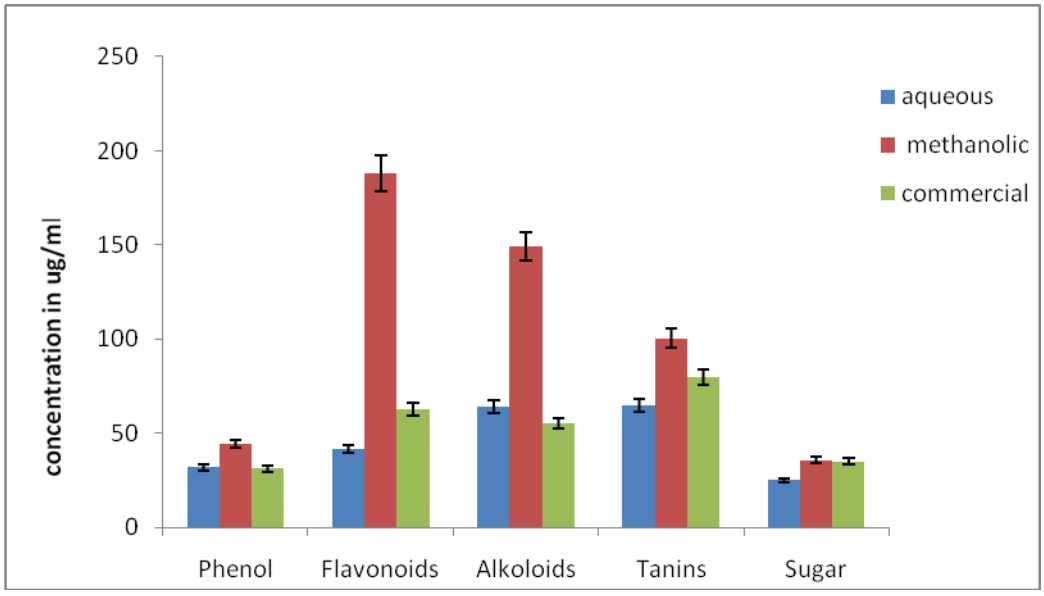
RESULTS AND DISCUSSION

Medicinal plants have been recognized as a potential source of natural antioxidants throughout the world owing to their natural origin, cost effectiveness and lesser side effects. Among the various medicinal plants, few endemic species are of particular interest as raw materials or preparations containing phytochemicals with significant antioxidant capacities [10]. Antioxidant property of phytochemicals arise from

their high reactivity as hydrogen or electron donors to the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions by termination of the Fenton reaction [1].

In the present investigation, antioxidant activities of methanolic and aqueous extracts of *Morinda citrifolia L* was compared to commercially available noni juice. They were found to contain a number of phytochemicals such as flavonoids, phenols, alkaloids, tannins and small amount of sugars.

Figure 1: Phytochemical constituents in *Morinda citrifolia L* extracts



(The results obtained were expressed as Mean ± S.D. of triplicates).

The *in vitro* methods for evaluation of antioxidant activity have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. These methods are popular due to their high speed and sensitivity [11].

Table 1: Total chlorophyll and carotene content in *Morinda citrifolia* fruit extract

PLANT *Morinda citrifolia L* fruit extract	Total Chlorophyll (g/l)	Chlorophyll a (g/l)	Chlorophyll b (g/l)	Carotene (g/l)
Aqueous	0.017±0.002	0.005±0.001	0.012±0.001	0.419±0.05
Methanolic	0.027±0.002	0.010±0.001	0.017±0.001	0.715±0.05
Commercial	0.016±0.002	0.006±0.001	0.010±0.001	0.512±0.05

(The results obtained were expressed as Mean ± S.D. of triplicates).

Both aqueous and methanolic extracts exhibited DPPH anion scavenging power but the methanolic extract showed a far higher scavenging property. Commercial Noni had less DPPH activity in comparison to both. DPPH scavenging activity of the extracts can be correlated to the presence of flavonoids [1, 9].

The NO scavenging activity in *Morinda citrifolia L* extracts was estimated to be 100±0.57 µg/ml ascorbic acid equivalent in methanolic extract and 58.66±0.52 ug/ml ascorbic acid equivalent in aqueous extract and 73±0.50 ug/ml in commercial extract, indicating a significant difference between three fruit extracts. Nitric oxide radical scavenging activity is correlated to the presence of phenolic compounds like flavonoids [12].

The FRAP was estimated to be 46.3 ±1.52 µg/ml ascorbic acid equivalent in methanolic extract,18.52 ±0.25 µg/ml aqueous extract and 33.1±0.5 µg/ml ascorbic acid equivalent in commercial noni juice. The FRAP activity is correlated to catechin, ferulic acid and total phenols which are present in fruit [1, 10].

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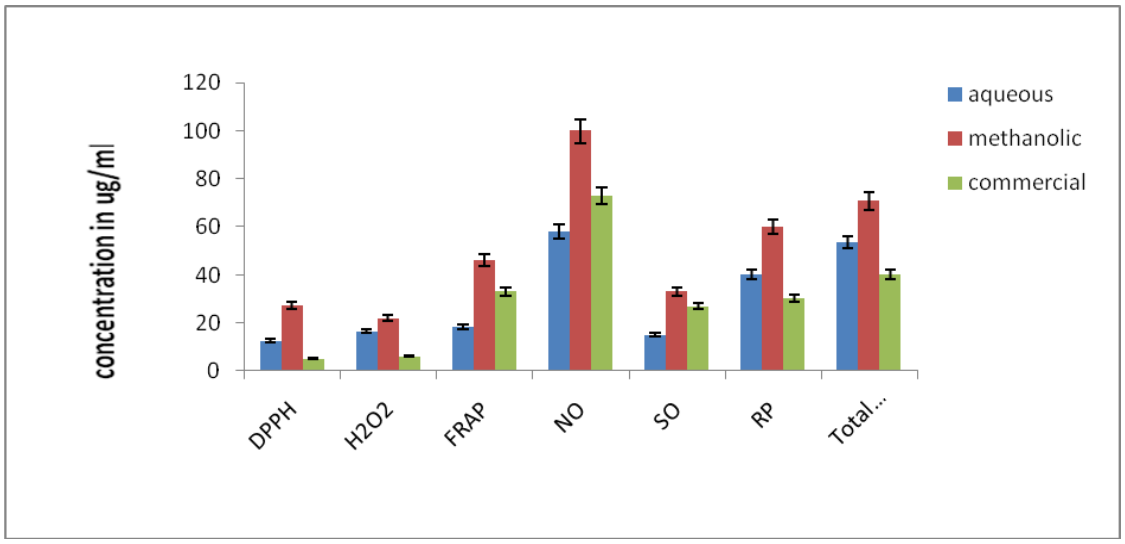
The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [8]. Reducing power is the measure of the reductive ability of antioxidant and it is evaluated by the transformation of Fe3+ to Fe2+ in the presence of extracts.

Figure 2: Antioxidant activity of *Morinda citrifolia* fruit extracts



(The results obtained were expressed as Mean ± S.D. of triplicates

Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [12]. The Reducing power was found to be higher in methanolic extract than in commercial and aqueous extract. The reducing power is mainly correlated to the presence of reductones like ascorbic acid [13]. Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress [1]. The SO scavenging activity was estimated to be 33.16 ±3.8µg/ml ascorbic acid equivalent in methanolic extract and 15.6 ± 2.6 µg/ml ascorbic acid equivalent in aqueous extracts. SO scavenging activity is correlated to total flavonoids [1,14].

Human beings exposed to H_2O_2 indirectly via the environment with intake from leaf crops contributing most to this exposure. Hydrogen peroxide enters the human body through inhalation of vapour or mist and through eye or skin contact [16]. In the body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\cdot}$) that can initiate lipid peroxidation and cause DNA damage. Antioxidants scavenge hydroxyl radicals [17]. The H_2O_2 scavenging activity in fruit extract was estimated to be 22 ±0.17 µg/ml ascorbic acid equivalent in methanolic extract,16.5 ±0.17 µg /ml ascorbic acid equivalents in aqueous extract and 6.12±0.11 µg/ml indicating a significant scavenging activity. Hydrogen peroxide radical scavenging activity is correlated to the presence of total phenols [7].

Total antioxidant activity is expressed as the–equivalents of ascorbic acid. Total antioxidant activity was found to be 70.7±0.005 µg/ml ascorbic acid equivalent in methanolic extract 53.6±0.17µg/ml ascorbic acid equivalents in aqueous extract and 40±0.07 µg/ml in commercial noni juice.

CONCLUSION

The current study shows that *Morinda citrifolia* fruit extracts possess significant amount of phytochemicals and *in vitro* antioxidant activity. A significant linear relationship between antioxidant activity and phytochemicals, that are responsible for the *in vitro* antioxidant property of the fruit extracts, supported this observation. The methanolic extract demonstrated to be a better candidate in terms of its antioxidant capacity. Analyzing the results, it is clear that the methanolic extract is capable of separating and solubilises both polar and nonpolar compounds and thus account for the plethora of bioactive compounds in comparison to that of aqueous and commercially available fruit extract.

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REFERENCES

[1] Ravi NV, Kanchanlata S, Thankamani M. Res J of Pharma Biol Chemical Sci 2012; 3:605-13
[2] Noor Hafizah,Maskat M Y.et al. Intl Food Res J 2012;9 (3): 1211-1215
[3] Micronutrient and phytochemical screening of a commercial Morinda citrifolia juice and a popular blackcurrant fruit juice commonly used by Athletes in Nigeria World Rural Observations 2015;7(1):40-48
[4] Z Kharis, LP Azimahtol , A Normah. J Trop Agric Food Sci 2010;38(2):203–209.
[5] Sridevi N, Changam S, S, Kotturam M C. Asian J Pharma Clinical Res 2012; 5:179-81.
[6] Samiraj R, Muthubalaji R, et al. Int J Pharma and Pharmaceutical Sci 2012;4(5): 473-476
[7] Blazovics A, Lugasi A, Szentmihalyi K, Kery A. Acta Biological Szegediensis 2003; 47: 99- 102.
[8] Samuel B, Elizabeth, K. Murugesan. Int Res J Pharmaceuticals 2012; 2,(4):, 106-111.
[9] Praveen K,Awagan B. J Eng Sci and Tech 2007;2(1); 70 – 80.
[10] Ravi N V, Kanchanlata S, Thankamani M. Free Radical and Antioxidant 2012;2(1):31-36.
[11] Chandha S, Dave R. African J Microbiol Res 2009; 3: 981-996.
[12] Hariom S, Saswata B. IJPSR 2015; 6(4): 1342-1350
[13] Vennila S, Brindha D. Int J Pharm and Pharmaceutical Sci2004;6(4): 55-59
[14] Rohit Y,Saurabh R. World J of Pharmaaceutical Res.2012;1(4);41-58.
[15] Jayaraman J. Estimation of Chlorophyll and Carotenoid. In Laboratory Manual in Biochemistry 2011, New Age International, New Delhi,
[16] McCune L, Johns T. J Ethnopharmacol 2002; 82:197-205.
[17] SN Kishore Kumar, M Suresh, S Ashok. Int J Curr Microbiol App Sci 2014; 3(2): 28-42.