Original Article

Comparative antioxidant evaluation of three indian cardio-protective medicinal plants in vitro studies

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ABSTRACT

A number of Indian medicinal plants have been used for thousands of years in the management of cardiovascular diseases such as atherosclerosis, hypertension. Oxidative damage remains as the important factor for the development of cardiovascular diseases. A comparative antioxidant evaluation of three cardio-protective plants pays the better way to fix the proportion of individual plants in the development of new formulation. With this aim, the antioxidant activity of three medicinal plants is evaluated in the *in-vitro* condition. 70% ethanolic extract of *Nelumbo nucifera* (NN), *Withania somnifera* (WS) and *Terminalia arjuna* (TA) were tested for their antioxidant activity and IC_{50} values were determined. To confirm the potent antioxidant plant their phenol, tannin, Vitamin C, Vitamin E and carbohydrate content were also estimated. HPTLC profile ofall the three extracts were also taken. The results from the ABTS and nitric oxide radical scavenging assay showed that IC_{50} value of all the three extracts were similar. Whereas in antilipid peroxidation assay NN has lower IC_{50} value than other two extracts. Tannin, Vitamin C, Vitamin E and carbohydrate content of TA was found to be high. In NN, Total phenolic content was found to be high. In conclusion, NN is more potent in exhibiting antioxidant activity than the other two extracts.Whereas free radical scavenging activity is exhibited by all the three extracts equally. Along with NN, TA is more potent in phytoconstituents content. Thus increasing the content of NN or TA in the formulation can increase the potency in cardiovascular disorder.

KEYWORDS: ABTS, nitric oxide radical, tannin, HPTLC, Vitamin C.

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INTRODUCTION

Free radicals are highly responsible for the development of various diseases including cardiovascular disease [1]. Various phenolic compounds which are widely distributed in food and medicinal plants are versatile antioxidants against reactive oxygen species that cause cell damage and are implicated in many diseases [2]. Polyphenol compounds are reported to be a good source of natural antioxidants [3]. Besides the traditional resources used for antioxidants, many plant species have been investigated in the search for natural antioxidants [4].

Nelumbo nucifera (Geartn) (NN) belongs to the family *Nelumbonaceae* is a handsome aquatic herb, with stout, creeping rhizome is found through out India. Ethanolic extract from seeds exhibits antioxidative and hepatoprotective effects [5]. Ethanolic extract of rhizome reduces blood sugar level [6]. Ethanolic extract of rhizome exhibits various psycho-pharmacological effects like reduction in spontaneous activity, decrease in exploratory behavioral pattern by the head dip and Y-maze test, reduction in muscle relaxant activity by rotarod, 30° inclined screen and traction test and potentiated the pentobarbitone induced sleeping time in mice [6]. It causes reduction of triglyceride and cholesterol level.

Withania somnifera (L) (WS) belongs to the family *Solanaceae*, is an erect, branched, unarmed shrub grows throughout the drier parts and sub-tropical India. It exhibits adaptogenic and cardioprotective activity in rats and frogs [7]. The hypotensive effect of WS might be due to autonomic ganglion blocking action and a depressant action on the higher cerebral centers [8].

Terminalia arjuna (TA) (belongs to the family *Combretaceae* is a large tree with smooth and thick bark found throughout the greater parts of India. It exhibits antioxidant and hypocholesteremic effects [9]. Early physiological studies carried on the isolated frog and rabbit heart revealed that the bark of TA had cardiotonic and stimulant actions [10]. It was subsequently found that intravenous administration of the glycoside, obtained from the bark of TA resulted in rise in blood pressure [11]. Interestingly the aqueous extract of the bark in isolated rat atria demonstrated positive inotropic activity [12]. This was again confirmed in subsequent work where aqueous extract of the bark in isolated rat atria produced inotropic action which was abolished by propanolol and cocaine [13].

Nowadays, pharmaceutical companies are seeking for polyherbal formulations mainly for their less toxic and high synergestic effect. A study should be undertaken, to find out the more potent herb in invitro condition and then the result might be useful to the Pharmaceutical industry to fix the concentration of individual herb in the formulation. With this aim three Indian medicinal plants which are reported and traditionally used for the cardiovascular diseases. (NN, TA, WS) are selected for the present study. The *In vitro* antioxidant activity of the 70 % ethanolic extract of all the three extracts were carried out. In order to find out the more potent extract their HPTLC profile along with the phytoconsitutents content were estimated.

MATERIALS AND METHODS

Chemicals and reagents: 2,2'-Azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) was purchased from Sigma, MO, USA. The other chemicals used were of analytical grade.

Collection and identification of herbs: Earlier references reveal that flower and seed of NN, root of WS and bark of TA were cardioprotective in nature [14,15]. So that the same parts of NN, WS and TA were collected respectively from Nagercoil, Madurai and Thirunelveli, Tamilnadu, Inida. They were identified and authentified and stored in Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Tamilnadu, India. The voucher number is 0092 for NN, 0064 for WS and 0094 for TA.

Preparation of extracts: The collected plant materials were cleaned, shade dried for 15 days and coarsely powdered in grinding mill. 70 % ethanolic extracts (70:30 Ethanol: water) of all the three medicinal plants were prepared as follows. 100 gm each of the dried plant was soaked in 70 % ethanol for 3 days in room temperature (26- 28°C). The solvent was evaporated under reduced pressure and then lyophilized. The yield of extract for NN, WS and TA was calculated as 8.49 %, 3.83 % and 27.7 % respectively. The samples were dissolved in 70 % ethanol and used for further in-vitro antioxidant activity.

$ABTS^{+\cdot}$ radical cation decolorization assay: ABTS radical cation decolorisation assay was estimation by following the method of Re et al.,[16]. ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulfate and the mixture was allowed to stand in dark at room temperature for 24 h before use. After 24 h the ABTS solution was diluted with 70 % ethanol, to an absorbance of 0.70 (± 0.02) at 745 nm. One milliliter of diluted ABTS solutions were mixed with 1.0 ml of extract containing different concentration of extract and the percentage inhibition was calculated against control. The control tubes contain 1.0 ml of ABTS and 1.0 ml of 70 % ethanol. Water was used as blank.

% Inhibition = (Control O.D. – Test O.D./ Control O.D.)X100

IC_{50} value is 50 % inhibition of ABTS decolorisation and this was calculated using GraphPad Prism version 5.0. Triplicate was carried out and the mean value was used for IC_{50} determination.

Lipid peroxidation:

Preparation of rat Heart homogenate: Rats weighing 200-250 g were sacrificed by decapitation and whole heart was dissected out and homogenized (100 mg/ml) in ice-cold phosphate buffer (0.1 M, pH 7.4) medium and used as a source of polyunsaturated fatty acids for determining the extent of lipid peroxidation. The tissue homogenate was centrifuged at 800 g for 15 min at 4°C and the supernatant was used for further study. Control tube contains 25 ìl of heart homogenate along with 25 ìl of ferrous sulphate (0.15 M). Different concentration of extracts were mixed with 25 ìl of heart homogenate along with 25 ìl of ferrous sulphate and incubated in a shaking water bath at 37°C for 40 min in open test tubes. Stock solutions of different concentrations of the extracts were diluted to 1.0 ml with buffer and were added to the homogenate at the beginning of the incubation period. In case of control experiments, volume adjustments were done with phosphate buffer. Incubations were terminated by the rapid addition of 3.5 ml 20% (v/v) acetic acid. All the tubes were subjected for Thiobarbituric acid Reactive substances assay.

Thiobarbituric acid reacting substances (TBARS) assay: After incubation was terminated, 600 µl of 0.5% Thiobarbituric acid (TBA) in 20% (v/v) acetic acid (pH 3.5) was added to each sample. The tubes were then incubated at 85°C for 60 min. After cooling 50 µl of 10 % sodium dodecyl sulfate (SDS) was added and centrifuged at 5000 rpm for 10 min and the absorbance of the supernatant was determined at 532 nm [17]. The absorbance was recorded against blanks prepared in the same way as the experimental samples but without homogenate. Percentage inhibition and IC_{50} values were determined as mentioned in ABTS radical cation decolorization assay. The experiment was repeated thrice.

Determination of Nitric Oxide Radical Scavenging Activity: Nitric oxide radical scavenging activity was determined by following the method of Govindharajan *et al*., (2003) [18] with slight modification. Sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) was mixed with 3.0ml of different concentrations (10 – 320 µg/ml) of the drugs dissolved in the suitable solvent systems and incubated at 25 °C for 300 min. The samples from the above were reacted with Greiss reagent (1% sulphanilamide, 2 % H_3PO_4 and 0.1 % napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm.

$$\text{NO scavenged (\%)} = \frac{(\text{Acont—Atest})}{\text{Acont}} \text{ X } 100$$

Where 'Acont' is the absorbance of the control reaction and 'Atest' is the absorbance in the presence of the sample of the extracts.

Determination of total polyphenol content: Total polyphenols were determined by Folin–Ciocalteu procedure Slinkard and Singleton, 1977 [19]. 70 % ethanolic extract of NN was dissolved in the same solvent. 0.5 ml of sample was mixed with 0.25 ml of Folin–Ciocalteu reagent and 1.25 ml 20% aqueous sodium carbonate solution, samples were vortexed and absorbance of blue colored mixtures recorded after 40 min at 725 nm against a blank containing 0.5 ml of 70 % ethanol, 0.25 ml of Folin–Ciocalteu reagent and 1.25 ml 20% aqueous sodium carbonate solution. The calibration curve was prepared from Phenol. The amount of total polyphenols was mentioned as mg of phenol/g of extract. Measurements were done in triplicate.

Quantification of tannins: Tannin content of 70 % ethanolic extract of NN was determined by flowing the method of Okwu Okwu, 2005 [20]. 500 mg of 70 % ethanolic extract was weighed in to 100 ml bottle. 50 ml of the same solvent was added and shaken for 1 hr in a mechanical shaker. This was filtered in to a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out in to a tube and mixed with 3 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M Potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 630 nm wavelength. A blank sample was prepared and the color developed was read at the same wavelength. Different concentration of tannic acid was used as standard. The final concentration of tannins was represented as mg/gm of extract. Measurements were done in triplicate.

Estimation of Total Carbohydrate: Total carbohydrate content of plant material was

estimated by following the method of Dubois [21]. 50 mg of sample was ground well with 2-3 ml of 5 % TCA. To the de-proteinized supernatant 10 ml of 45 % ethanol was added to precipitate the polysaccharides. After setting it to stand overnight in cold, the tube was centrifuged for 10 minutes at 4000 rpm. The dried precipitate was analyzed for total carbohydrate by dissolving in 2 ml of 1N NaOH. The sample was made up to 1ml with water. 1 ml of 5 % Phenol and 5ml of concentrated sulphuric acid were added. The mixture was mixed thoroughly with a glass rod. The solution was allowed to stand for 10 minutes at room temperature and its optical density was read at 490 nm in a spectrophotometer and standard graph was prepared by using different concentration of D-Glucose ranging from 10 to 100 µg/ml.

Estimation of Vitamin C: Vitamin C in the plant was estimated according to the method of Sarojini and Nittala 1999 [22] with slight modifications. 1.0 gm of plant material was soaked in 75% ethanol for 24 hours. The extract was filtered. The filtrate was used as sample for the estimation of vitamin C. 0.1 ml of sample was made up to 1.0 ml with water. Different concentrations of ascorbic acid from 4 – 20 µg/ml were used as the standard. Blank contains 1.0 ml of water. 0.5 ml of dinitro phenyl hydrazine (0.2%) was added to all the tubes including blank, test and standard test tubes. Incubate all the tubes at room temperature for 3 hours. 2.5 ml of 85% Sulphuric acid was added to all the tubes. The absorbance was measured at 520nm

Estimation of Vitamin E by Dipyridyl Method: Vitamin E in the plant was estimated according to the method of Jayashree et al., (23)1985) with slight modifications. 1.0 gm of plant material was soaked in 100 ml of ethanol: petroleum ether (1.5:2.0) for 24 hours. The petroleum ether fraction was separated and evaporates to dry. The precipitate formed was mixed with 5.0 ml of ethanol. 0.2 ml of extract was made up to 1 ml with ethanol. To this, 0.2 ml of 2,2', µ-dipyridyl reagent (0.2 %), 0.2 ml of ferric chloride (0.5 %) solution and 2.0 ml of butanol were added. For blank, 0.2 ml of ethanol, 0.2 ml of 2,2'- dipyridyl reagent, 0.2 ml of ferric chloride and 2.0 ml of butanol were added The red colour developed was read exactly after 15 min. at 520 nm. Different concentrations of á-tocopherol were used as standard. The calculated tocopherol was mentioned as µg/100 g of plant material.

HPTLC fingerprinting of all the three extracts: Chromatography was performed on a preactivated (110^{0}C) silica gel HPTLC plate silica 60GF$_{254}$. 70% hydroalcoholic extract of all the three extracts dissolved in methanol at the concentration of 10 mg/ml was applied on the silica gel coated plate with an automatic applicator Linomat 5 with N_2 flow (CAMAG, Switzerland). The number of bands for each sample is 2. Initially the TLC chamber was saturated with Butanol:acetic acid: water (4:1:1) for NN, Chloroform : methanol (8:2) for WS and TA for 1 hour. The sample applied plate was developed using the mobile phase upto 80 mm of the plate. The HPTLC runs were in laboratory conditions of 25 ± 5^{0}C and 50 % relative humidity. After development the plate was withdrawn and air dried and spots were visualized in UV light (UV cabinet, CAMAG, Switzerland). The plate was photodocumented at 254 and 366 nm. The plate was scanned in the scanner, CAMAG, Swizerland. Scanning conditions for NN are Position of first track – 10.3 mm, distance between tracks – 10.0 mm, scan start position Y- 10.0 mm, scan end position – 84.0 mm, wavelength – 366 nm, for WS are Position of first track – 10.8 mm, distance between tracks – 11.4 mm, scan start position Y- 10.0 mm, scan end position – 82.0 mm, wavelength – 366 nm and for TA are Position of first track – 9.6 mm, distance between tracks – 11.4 mm, scan start position Y- 6.0 mm, scan end position – 85.0 mm, wavelength – 366 nm,. Other scanning conditions like slit dimensions – 6.0X0.45 mm, scanning spped – 20.0 mm/s, data resolution – 100 ìm/step, lamp – D2 & W are same for all the three extracts.

RESULTS

Data of Table 1 shows the antioxidant activity of NN, WS and TA in different methods. Along with the concentration of extracts the percentage inhibition of TBARS formation, NO radical scavenging and ABTS$^{+\cdot}$ radical cation decolorization were found to be increased (Table 1). The IC_{50} value calculated using Graphpad software

version 5.0 reveals that there is no difference between all the three extracts in ABTS and NO radical scavenging assays. Whereas, NN prevent the TBARS formation at the lower concentration itself followed by WS and TA (Fig 1).

Fig 2 reveals that Phenolic content of NN is high followed by TA and WS. Moreover, TA exhibits the presence of higher concentration of all the other phyto-constituents like tannin, carbohydrate, Vitamin C and Vitamin E.

HPTLC profile of WS showed the presence of 8 peaks followed by NN with 7 peaks. The % Area of the peak with maximum Rf value in all the three extracts was found to be high (Table 3, Fig 2,3 and 4). The HPTLC profile of TA showed the presence of only 3 major peaks (Table 3, Fig 4).

Parameters	Extract	% Inhibition				
Antilipid peroxidation	Concentration (µg/ml)	100	200	300	400	500
	NN	37.4 ± 3.0	50.88 ± 5.0	65.09 ± 6.2	76.5 ± 6.5	86.7 ± 7.3
	WS	37.7 ± 3.0	46.6 ±4.0	59.2 ± 5.0	77.4 ± 6.2	89.3 ± 7.3
	TA	26.2 ± 2.3	36.6 ± 2.5	55.9 ± 3.6	68.2 ± 5.6	87.9 ± 6.8
Nitric oxide radical scavenging		% Inhibition				
	Concentration (µg/ml)	100	200	300	400	500
	NN	31.5 ± 2.8	40.1 ± 3.5	53.0 ± 4.2	60.4 ± 5.2	68.1 ± 5.9
	WS	24.4 ± 1.9	35.7 ± 3.2	52.9 ± 4.0	63.6 ± 5.2	76.8 ± 6.3
	TA	28.5 ± 2.1	40.7 ± 3.5	55.4 ± 4.5	70.5 ± 5.9	85.7 ± 6.8
$ABTS^{+}$ radical cation decolorization		% Inhibition				
	Concentration (µg/ml)	20	40	60	80	100
	NN	21.4 ±2.3	35.6 ± 5.9	48.5 ± 9.8	56.6 ± 8.7	72.6 ± 9.6
	WS	11.2 ± 2.5	35.6 ± 6.5	45.6 ± 8.9	68.9 ± 5.8	79.8 ± 9.8
	TA	33.5 ± 2.5	45.6 ± 6.5	54.2 ± 8.7	65.6 ± 9.8	75.8 ± 8.5

Table 1: Effect of NN (*Nelumbo nucifera),* WS (*Withania somnifera)* and TA (*Terminalia arjuna*) on *In-vitro* antioxidant assay.
(Values Are Mean ± SD)

Table 2: Total Phenolic, tannin, Vitamin C, Vitamin E and carbohydrate content of NN (*Nelumbo nucifera),*WS (*Withania somnifera)* and TA (*Terminalia arjuna*).

S. No.	Plants	Total Phenolics (mg/100 gm)	Total Tannins (mg/100 gm)	Vitamin C (mg/100 gm)	Vitamin E (mg/100 gm)	Total Carbohydrate (mg/100g m)
1	NN	10.20 ± 0.1	4.30 ± 0.3	0.36 ± 0.2	0.42 ± 0.2	672.0 ± 2.3
2	WS	2.86 ± 0.3	3.20 ± 0.2	0.26 ± 0.2	0.46 ± 0.6	748.0 ± 3.2
3	TA	8.05 ± 0.2	5.10 ± 0.1	1.47 ± 0.1	0.58 ± 0.3	1154.0 ± 5.3

Extract	Track number	Total number of peaks	Peak number/Rf	Peak number/% area
NN	1	7	1/0.33, 2/0.42, 3/0.51, 4/0.63, 5/0.67, 6/0.74, 7/0.89	½.08, 2/3.46, 3/18.21, 4/8.12, 5/6.28, 6/3.86, 7/57.99
	2	8	1/0.32, 2/0.41, 3/0.46, 4/0.53, 5/0.61, 6/0.65, 7/0.74, 8/0.88	1/1.3, 2/4.94, 3/3.99, 4/20.83, 5/8.97, 6/7.29, 7/6.03, 8/46.65
WS	1	6	1/0.34, 2/0.64, 3/0.65, 4/0.74, 5/0.85, 6/0.89	1/0.61, 2/3.02, 3/2.67, 4/7.14, 5/30.40, 6/56.15
	2	8	1/0.34, 2/0.46, 3/0.54, 4/0.63, 5/0.72, 6/0.76, 7/0.84, 8/0.88	1/0.65, 2/1.35, 3/1.98, 4/5.83, 5/10.56, 6/8.03, 7/20.77, 8/50.83
TA	1	3	1/0.51, 2/0.69, 3/0.79	1/12.87, 2/11.54, 3/29.77
	2	3	1/0.50, 2/0.68, 3/0.78	1/12.38, 2/16.06, 3/40.57

Table 3: Rf value and % Area of HPTLC plate for all the three extracts.

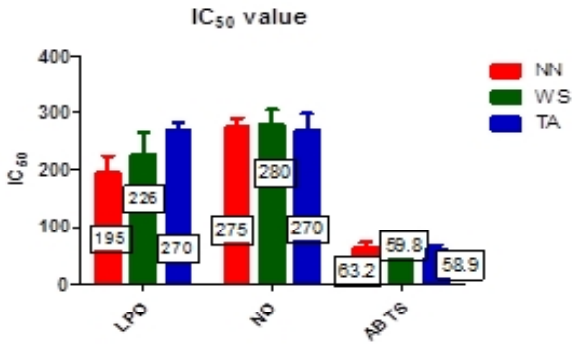


Fig. 1: IC_{50} Value of NN (*Nelumbo nucifera*), WS (*Withania somnifera*) and TA (*Terminalia arjuna*) in Anti-lipid peroxidation assay, Nitric oxide radical scavenging assay and $ABTS^{+\bullet}$ radical cation decolorisation assay.

Fig. 2: HPTLC profile of NN. A – Photodocumentation of HPTLC plate at 254 nm. B- Photodocumentation of HPTLC plate at 366 nm. C - Peak display of second track.

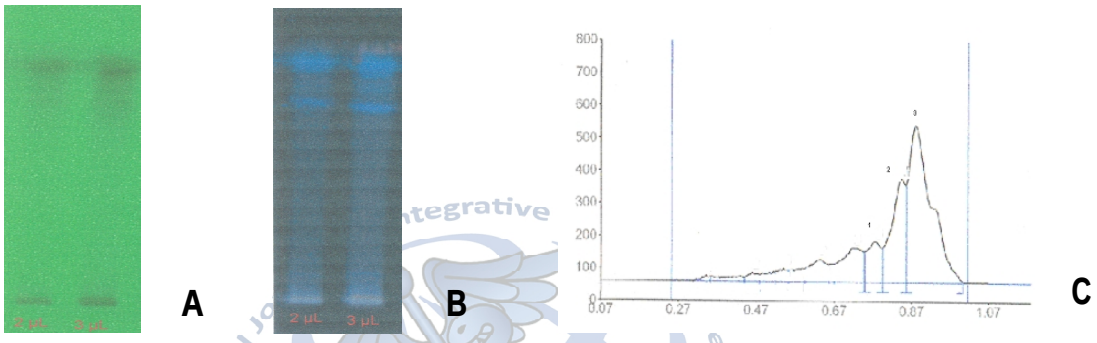


Fig. 3: HPTLC profile of WS. A – Photodocumentation of HPTLC plate at 254 nm. B- Photodocumentation of HPTLC plate at 366 nm. C - Peak display of second track.

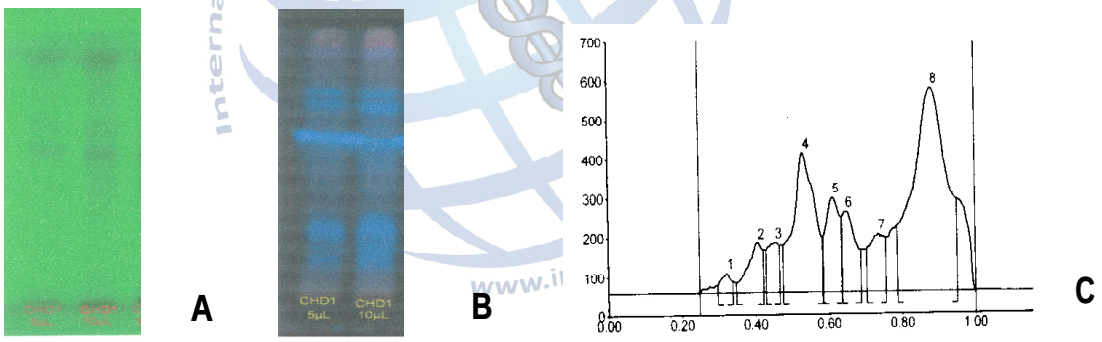
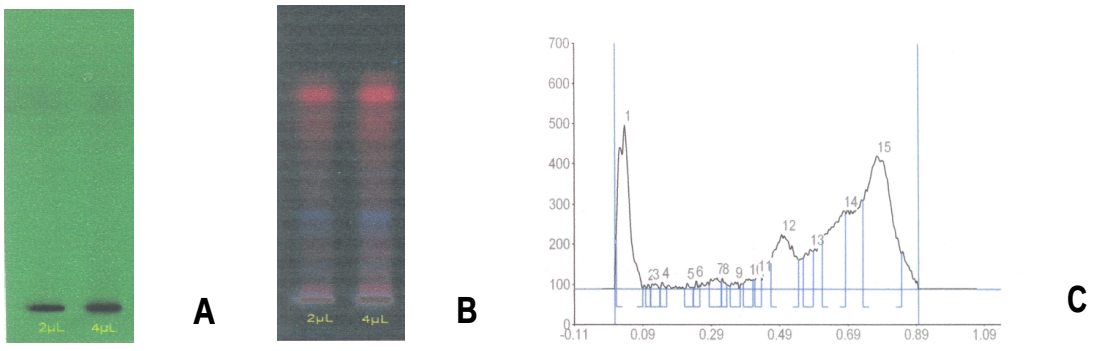


Fig. 4: HPTLC profile of TA. A – Photodocumentation of HPTLC plate at 254 nm. B- Photodocumentation of HPTLC plate at 366 nm. C - Peak display of track.



DISCUSSION

Antioxidant may be defined as "a substance when present at lower concentrations compared with those of an oxidizable substrate such as fats, proteins, carbohydrates or DNA, significantly delays or prevent oxidation of substrate. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids [24]. In general, antioxidant systems either prevent these reactive species

from being formed, or remove them before they can damage vital components of the cell [25].

Various extracts were tested in this study for their radical scavenging activities on two different radicals like NO, ABTS. The ABTS system provides information on the reactivity of a test compound with a stable free radical. The ABTS radical cation decolorisation assay is a widely used method to measure the total antioxidative status of various biological specimens because of the good reproducibility and quality control [26]. This method apply decolorisation assay to monitor the decrease in their absorbance during the reaction. For example the ABTS radical absorb at 734 nm. When an antioxidant is added to the radical, there is a degree of decolorisation owing to the presence of antioxidant which reserves the formation of the ABTS radical cation. The scavenging activity of cation is shown by increasing the concentration of extracts and decreases the color of ABTS.

NO a vasodilator, reacts with superoxide radical results in the formation of peroxynitrite. NOS inhibitors are important drugs for treating various diseases related with inflammation [27]. In our present experiment, Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide [28], which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide [29]. All the three extracts were found to scavenge the nitric oxide with increasing the concentration. Similar IC_{50} value of all the three extracts reveals that, all extracts are similar in their NO radical scavenging activity.

Anti-lipid peroxidation activity of extracts was found to be increased with increasing the concentration. The inhibition could be caused by the absence of ferryl–perferryl complex or by scavenging of OH radical or superoxide radicals or by changing the ratio of Fe^{3+}/Fe^{2+} or by reducing the rate of conversions of ferrous to ferric or by chelating the iron itself [30].

NN extract exhibits lower IC_{50} value than other extracts which reveal that NN is more potent in the antioxidant activity. This effect might be due to the presence of higher concentration of phenol (Table 2)

Total phenolic content of all the three extracts were calculated in the present study and their values are mentioned in the Table 2. Polyphenolics is a highly inclusive term that covers many different subgroups of phenolic acids and flavonoids. More than 5000 polyphenolics, including over 2000 flavonoids have been identified, and the number is still growing. Many of the phenolics are often associated with sugar moieties that further complicate the phenolic profiles of plants. Polyphenols are especially important antioxidants, because of their high redox potentials, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelating potential. The antioxidant activity of the dietary polyphenolics is considered to be much greater than that of the essential Vitamins, therefore contributing significantly to the health benefits of fruits [31].

Total carbohydrate and glycosides play an important role in immunomodulatory reactions and their free radical scavenging activity has been reported earlier by Morelli et al., (2003) [32]. This made us to estimate the level of Total carbohydrate in different extracts. TA has shown to consist of higher concentration of glycosides. Presence of carbohydrate in all the three extracts might prevent the formation of ferryl-perferryl complex.

The tannin concentration of TA was found to be high (Table 2). The high tannin content could be partly responsible for bitter principle associated raw plant. Flavonoids and tannins are reported to inhibit superoxide radical generation [33]. That NO promotes IL-1â induced cartilage destruction through the activation of MMP-3 and -9 [34]**.** No significant difference between the IC_{50} value of NN, TA and WS might be due to the presence of tannin in all the three extracts.

Vitamin C concentration of TA was found to be higher among all the three extracts (Table 2).

Vitamin C is one of the most potent reducing agents for biological systems. When Vitamin E turns into tocopheroxyl radical this reacts with Vitamin C that restores tocopherol [35]. Anti-lipid peroxidation activity of all the three extracts might be due to the presence of Vitamin C.

Vitamin E concentration of TA was found to be high (Table 2, Fig 1). Cherubini et al. (2001) [36] suggested that maintaining proper Vitamin E status is important to avoid increased risk for atherosclerosis with advanced age. The antioxidant activity of all the extracts might be due to the presence of Vitamin E.

SUMMARY AND CONCLUSION

The antioxidant activity of NN, WS and TA was reported to exhibits the antioxidant activity. The free radical scavenging activity of all the three extracts was similar. Whereas, NN exhibits more potent anti-lipid peroxidation assay than other two extracts. Since all the three extracts are exhibiting potent antioxidant activity, a poly herbal formulation can be produced by mixing the three extracts with NN alone in higher amount to potent the biological activity. The presence of phenol, tannin, carbohydrate, Vitamin C and Vitamin E in NN, WS and TA shows that these plants are potent neutraceutical agent.

REFERENCES

[1]. Halliwell, B., and Gutteridge, J.M.C.,7 Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J. 1984;219:1-14.

[2]. Pietta, P.G., Flavooids as antioxidants. J.Nat. Prod. 2000;63:1035–1042.

[3]. Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W. and Riechel, T.L., High molecular weight plant phenolics (tannis) as biological antioxidants. J. Agric. Food Chem.1998;46:1887–1892.

[4]. Banias, C., Oreopoulou, V. and Thomopoulos, C.D., The effect of primary antioxidants and synergists on the activity of plant extracts in lard. J. Am. Oil Chem. Soc.1992;69:520–524.

[5]. Sohn, D.H., Kim, Y.C., Oh, S.H., Park, E.J., Li, X. and Lee, B.H., Hepatoprotective and free radical scavenging effect of Nelumbo nucifera. Phyto-medicine. 2003;10(2-3):165-169.

[6]. Pulok, K., Mukherjee, Kakali Saha, Pal, M. and Saha, B.P., Effect of Nelumbo nucifera rhizome extract on blood sugar level in rats. Journal of Ethnopharmacology 1997;58(3):207–213.

[7]. Pulok K, Mukherjee, Kakali Saha, Balasubramanian, R., Pal, M. and Saha, B.P. Studies on psychopharmacological effects of Nelumbo nucifera Gaerin. Rhizome extract. Journal of Ethnopharmacology 1996;54(2-3):63-67.

[8]. http://www.pfaf.org/database/search_ use.php?K%5B%5D=Cardiotonic&START=100

[9]. Jayant, N., and Dhuley., Adaptogenic and cardioprotective action of ashwagandha in rats and frogs. Journal of Ethnopharmacology. 2000;70(1):57–63.

[10]. Malhotra, C.L., Das, P.K., Dhalla, N.S. and Prasad, K., Studies on Withania ashwagandha, Kaul. III. The effect of total alkaloids on the cardiovascular system and respiration. Indian J Med Res.1981;49:448-460.

[11]. Gupta, R., Singhal, S., Goyle, A., Sharma, V.N., Antioxidant and hypocholesterolaemic effects of Terminalia arjuna tree bark powder randomized placebo controlled trial. The journal of The Association of Physicians of India 2001;49:233–235.

[12]. Ghoshal, L.M. Terminalia arjuna. Ph.D. thesis, Calcutta University, Calcutta, India 1909.

[13]. Ghosh, S. Annual report of the Calcutta School of Tropical Medicine. Institute of Hygiene and the Carmichel Hospital for Tropical Diseases, Calcutta, India 1926.

[14]. Karamsetty, M., Ferrie, T.J., Kane, K.A., Gray, A.I., Effects of an aqueous extract of Terminalia arjuna on isolated rat atria and thoracic aorta. Phytotherapy Research 1995;9:575–578.

[15]. Lakshmi-Chandra Mishra, Betsy, B., Singh, Simon Dagenais, BA. Scientific Basis for the Therapeutic Use of Withania somnifera (Ashwagandha):A Review, Alternative Medicine Review 2000;5(4):342-352.

[16]. Caius, J.S., Mhaskar, K.S. and Isaacs, M., A comparative study of the dried barks of the commoner Indian species of genus Terminalia. Indian Medical Research Memoirs 1930;16:51–75.

[17]. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C., Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. 1999;26:1231-1237.

[18]. Okhawa, H., Ohishi, N. and Yagi, K., Reaction of Linoleic acid by hydroperoxides with Thiobarbituric acid, Anal Biochem.1979;95:351.

[19]. Govindarajan, R., Vijayakumar, M. and Pushpangadan, P., Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. Journal of Ethnopharmacology 2005;99:165-178.

[20]. Slinkard, K., Singleton, V.L., Total phenol analysis: automation and comparison with manual methods. Am J Enol Vitic. 1977;28:49-55.

[21]. Okwu, D.E., Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. Int J Mol Med Adv Sci. 2005;1(4):375-381.

[22]. Dubois, M., Giles, M.K., Hamilton, J.K., Reber, P.A, and Smith, R. Colorimetric methods for the determination of sugar and related substances. Anal chem.1954;28:350-356.

[23]. Sarojini, Y. and Nittala S. Sharma., Vitamin C content of some macroalgae of Visakhapatnam, East coast of India. Indian J. Mar. Sci.1999;28:408-412.

[24]. Jayashree, V., Solimabi, and Kamat S.Y., Distribution of tocopherol (Vitamin E) in marine algae from Goa, West coast of India. Indian J, Mar. Scie. 1985;14:228-229.

[25]. Burits, M. and Bucar F., Antioxidant activity of Nigella sativa essential oil. Phytother Res. 2000;14:323-328.

[26]. Vertuani, S., Angusti, A. and Manfredini, S., The antioxidants and pro-antioxidants network: an overview. Current Parma Des. 2004;14:1677–1694.

[27]. Brand Williams, W., Cuvelier, ME. and Berset, C. Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technol 1995;28: 25-30.

[28]. Miller, M.J., Sadowska, K.H., Chotinaruemol, S., Kakkis, J.L. and Clark, D.A., Amelioration of chronic ileitis by nitric oxide synthase inhibition. Journal of Pharmacology and Experimental Therapeutics 1992;264:11-16.

[29]. Green, L.C., Wagner, D.A., Glogowski, J et al., Analysis of nitrate, nitrite, and (15N) nitrate in biological fluids. Anal Biochem.1982;126:131-138.

[30]. Marcocci, L., Maguire, J.J., Droy-Lefaix, M.T et al., The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Biochem Biophys Res Commun.1994;15:748-755.

[31]. Braughler, J.M., Duncan, C.A. and Chase, L.R., The involvement of ironin lipid peroxidation. Importance of ferrous to ferric ratio in initiation. Journal of Biological Chemistry. 1986; 261:10282.

[32]. Tsao, R. and Deng, Z., Separation procedures for naturally occurring antioxidant phytochemicals. Journal of Chromatography. 2004;812:85–99.

[33]. Morelli, R., Russo-Volpe, S., Bruno, N. And Scalzo, R.L., Fenton-dependent damage to carbohydrates: free radical scavenging activity of some simple sugars. Journal of Agriculture and Food Chemistry.2003;51;7418–7425.

[34]. Priyadarsini, K.I., Khopde, S.M., Kumar, S.S. and Mohan, H., Free radical studies of ellagic acid, a natural phenolic antioxidant, J. Agric. Food Chem. 2002;50:2200–2206.

[35]. Kato, H., Nishida, K., Yoshida, A., Takada, I., McCown, C., Matsuo, M., Murakami, T. and Inoue, H., Effect of NOS2 gene deficiency on the development of autoantibody mediated arthritis and subsequent articular cartilage degeneration, J. Rheumatol., 2003;30:247–255.

[36]. Bohm, F., Edge, R. L. E. J., McGarvey, D.J. and Truscott, T.G., Carotenoids enhance vitamin E antioxidant efficiency. J Am Chem Soc. 1997;119:621–22.

[37]. Cherubini, A., Zuliani, G., Costantini F, et al., High vitamin E plasma levels and low low-density lipoprotein oxidation are associated with the absence of atherosclerosis in octogenarians. J Am Geriatr Soc. 2001;49:651-54.

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