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Article

Assessment of antioxidant potential in seed extracts of *Nyctanthes arbor-tristis* L. and phytochemical profiling by Gas Chromatography-Mass Spectrometry system

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The present study has been carried out with the seed extracts of *Nyctanthes arbor-tristis* L. (Parijat) and evaluates its antioxidant potential and profiling the phytochemical constituents by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The antioxidant potential of the seed extracts was measured by four different *in vitro* assay like 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, superoxide anion free radical scavenging activity, ferric reducing antioxidant power (FRAP) and lipid peroxidation inhibition potential (LPIP) assay. The total phenol content (TPC) and total flavonoid content (TFC) were estimated. The ethyl acetate extract (EAE) of seeds showed potential DPPH free radical scavenging activity (EC_{50} 129.49±3.55µg/ml), superoxide anion radical (EC_{50} 969.94±8.03µg/ml) and LPIP (EC_{50} 452.43±5.07 µg/ml) activities. The total phenol content was maximum in aqueous extract (AQE) which was 201.00±0.20 µg/mg gallic acid equivalent. The EAE was rich with total flavonoid and it was found to be 34.50±0.40 µg/mg rutin equivalent. The EAE was subjected for phytochemical-profiling using GC-MS system. The presence of different phytoconstituents supports the medicinal value of the seeds. The results suggest that EAE constitutes a promising new source of novel compounds. Further, it can be used for isolation and purification of specific compounds which have good antioxidant activities and possess useful biological activities.

Keywords: Phytochemical profiling. Ferric reducing antioxidant power. Lipid peroxidation inhibition potential. Poly-unsaturated fatty acids. Reactive oxygen species.

INTRODUCTION

The use of plant as a medicine is as old as human civilization**.** The traditional systems of medicine such as unani and ayurveda have provided novel concepts and modalities in the healthcare area. Right from the ancient times, the importance of traditional medicines in the treatment of various infections and other chronic diseases is well documented (Pandey, Rastogi, Rawat, 2013). Use of medicinal plants is still a tradition followed by the ethnic community dwelling in the undulating planes and at the foothills of major forests of the globe. *N. arbor-tristis* L. (Parijat) of family Oleaceae is well known throughout India and all over the world and has a great impact on the health and general life. It is one of the most versatile mythological medicinal plant with high medicinal values in ayurveda and possess wide spectrum of biological activities. The plant based natural products are the main source for the drug discoveries since long times. Each part of the *N. arbor-tristis* has numerous ethnopharmacological values and utilized for folklore medicines (Sharma *et al*., 2021).The scientific literatures support the antioxidant (Mishra *et al*., 2016) and anti-

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plasmodial (Kumari *et al*., 2012) properties of the flower and leaf parts of the plant.

The fresh paste of the crushed powder of seeds along with aromatics is applied externally to cure many skin diseases such as skin eruption, dermatitis and scurfy affections of scalp, alopecia and also applied for cooling effect (Sharma *et al*., 2021). The glycerides of linoleic, oleic, lignoceric, stearic, palmitic and myristic acids have been reported from the seeds (Rahman, Roy, Shahjahan, 2011). The ethanolic extract of the *N. arbor-tristis* seeds has notable anti-helmintic properties (Das, Sasmal, Basu, 2010). The n-butanol fraction of *N. arbor-tristis* seed extract possesses anti-leishmanial properties (Tandon, Srivastava, Guru, 1991). The iridoid glycoside (arbortristoside-A) has been isolated from ethanolic extract of the seeds and possesses anti-inflammatory and anti-nociceptive activity (Das, Sasmal, Basu, 2008). The n-butanol fraction of seed extract of *N. arbor-tristis* is effective against encephalomyocarditis and semliki forest viruses and thus possesses anti-viral properties (Gupta *et al*., 2005).

Reactive oxygen species (ROS) is the radical derivatives of oxygen generated by the electron leakage from electron transport chain (ETC) and is considered the most important free radical in biological systems. The ROS are the harmful by products generated during the normal physiological and cellular functions and have negative impacts on the organism's health. The ROS includes superoxide radical ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), alkoxyl radical ($RO^{\bullet}$), lipid hydroperoxide (LOOH) and peroxynitrite ($ONOO^-$) which are generated as by product of biological reactions or exogenous factors (Karmakar *et al*., 2011; Apak *et al*., 2016). Antioxidants were considered as free radical scavenger by breaking chain reaction as well as by binding with the metal ion (Adjimani, Asare, 2015). In the recent year attention was given on natural antioxidants (Li *et al*., 2014). Natural antioxidant are safe than synthetic one (Liu *et al*., 2013). Natural antioxidant rich with phenol and flavonoids effectively scavenged the free radicals, chelates with the metals (Dzoyem, Eloff, 2015), and checked the progressive oxidative damages. Medicinal plants parts rich with antioxidants can be used to inhibit the damaging effects of the reactive oxygen species. Antioxidants fight against free radicals and protect against various diseases.

Some of the medicinal properties of the plant might be attributed due to its scavenging activity of reactive oxygen species (Kasote *et al*., 2015). However, there is no report on comparative antioxidant potential of crude extracts of seeds of *N. arbor-tristis*. Hence, the objective of this study was to determine the antioxidant potential of different seed extracts by DPPH and superoxide radical scavenging assays and to measure the total phenol and flavonoid content in seed extracts of *N. arbor-tristis*. Additionally, ferric reducing antioxidant potential (FRAP) and lipid peroxidation inhibition potential (LPIP) of extracts were investigated. Phytochemical profiling of the extract was performed to get an idea about chemical compounds present in the seeds using GC-MS analysis. To the best of our knowledge, there is no any systematic report regarding profiling of phytochemical constituents of the seed extracts of *N. arbor-tristis* using GC-MS analysis and evaluation its antioxidant properties.

MATERIAL AND METHODS

Seed collection site and preparation of extract

The seeds of *N. arbor-tristis* were collected from the ayurvedic garden of Banaras Hindu University, Varanasi, Uttar Pradesh, India (*NL25°16′23′′*and *EL82°59′50′′).* A voucher specimen (DG/15/124) was deposited in Department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University Varanasi. The seeds were washed under running tap water and dried at 25°C for 7 days. Dried seeds were grinded into course powder. Hot percolation extraction process was adopted for aqueous extract (AQE) preparation. For extraction 25.40 g of the powdered material was boiled in 250 ml distilled water for 30 minutes, kept for 3 days with intermittent shaking. Soxhlet extraction procedure was adopted for the preparation of ethanol extract (EE) and ethyl acetate extract (EAE) of seeds. For the preparation of EE and EAE, powdered seeds (25.40 g) were extracted in 250 ml of ethanol and ethyl acetate. Extracts were evaporated to dryness at 45°C with a rotatory evaporator. The dried semi-solid extracts were used for analysis and stored in refrigerator at 4°C for further studies.

Qualitative phytochemical analysis

Preliminary phytochemical analysis in different extracts was performed by the methods of Bhandary *et al.* (2012), Maria *et al.* (2018) and Gul *et al.* (2017).

Biochemical assay

The method of Brand-Williams, Cuvelier and Berset (1995) was used for the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging antioxidant analysis. The freshly prepared 3 ml methanol (0.004% w/v) DPPH solution was mixed in the seed extracts of various concentrations and the reaction mixture was incubated at 37°C for 15 minutes. The absorbance of solution was measured at 517 nm. Inhibition percentage of free radical was calculated by the formula given in Equation 1. Ascorbic acid was used as a reference control for antioxidant assay.

Inhibition percentage of free radical (%) = [(Absorbance of control-Absorbance of sample)/ Absorbance of control] ×100.... [Eq. 1]

Superoxide radical scavenging activity was measured by the method of Beauchamp and Fridovich (1971). The reaction mixture contained 0.01M phosphate buffer solution (PBS) (pH 7.8), 130 mM (w/v) methionine, 60 µM (w/v) riboflavin, 0.5 mM (w/v) EDTA, 0.75 mM (w/v) NBT, and 500 µl of test sample solution (seed extracts). The total volume of each reaction mixture was 3000 µl. The reaction mixture was exposed with fluorescent light (20W) for 6 minutes for initiating the reaction and later absorbance was recorded at 560 nm. The tubes containing reaction mixture were placed under dark condition and it served as control. The inhibition percentage of superoxide radical generation was measured by comparing the absorbance of the control and reaction mixture containing test sample by using the formula of equation 1. The 0.01M phosphate buffer solution was used as blank. The copper sulphate was used as reference control.

The FRAP of the extracts were determined by Oyaizu (1986) method. The different concentrations (50-1000 µg/ml) of the extracts was mixed with 2500 µl of sodium phosphate buffer (0.2M, pH 6.6) and 2500 µl of 1% potassium hexa-cyanoferrate (K_3 [Fe $(CN)_6$]) and incubated at 37 ºC for 20 min. After the addition of 2500 µl of 10% TCA, the mixture was centrifuged at 1000 rpm for 10 min. Then 2500 µl of upper layer was mixed with 2500 µl distilled water and 500 µl of 0.1% (w/v) $FeCl_3$ solution. The ascorbic acid was used as a reference control. FRAP was measured by determining the absorbance at 700 nm

Ohkawa, Ohishi and Yagi (1979) protocol was followed for the measurement of LPIP. Egg-yolk homogenates was used as source of lipid-rich media. The reaction mixture containing 250 µl of egg homogenate (10% in distilled water, v/v) and 50 µl of extracts were mixed properly and the volume was maintained up to 500 µl, by adding distilled water. Freshly prepared 25 µl of $FeSO_4$ (0.07Mw/v) was added in the test tube and incubated up to 30 minutes, to induce lipid peroxidation. Thereafter, 750 µl of 20 % (v/v) acetic acid (pH 3.5) and 750 µl of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulphate) and 25 µl 20% (w/v) TCA were added, vortexes, and then incubated on boiling water bath for 1 hour. After cooling, 3000 µl of 100 % 1-butanol was added to each tube, shaken vigorously and centrifuged at 3000 rpm for 15 minute. The absorbance of the organic upper layer was measured against 3000 µl butanol at 532 nm. The 50 µl distilled water present in the tube in place of the extracts was used as control. Ascorbic acid was considered as standard reference control.

Folin-Ciocalteu (FC) assay (Mc Donald *et al.*, 2001) was used for the determination of total phenol content (TPC) and were expressed as ***µg/mg*** gallic acid equivalents (GAE) through the gallic acid calibration curve. The method of Chang *et al.* (2002) was followed for the measurement of total flavonoid content (TFC) and was analyzed by rutin standard curve and was represented as ***µg/mg rutin equivalents*** (RE).

Gas chromatography mass spectrometry (GC–MS) analysis of EAE of seed

The compounds were analyzed using GC-MS (GC-MS-QP-2010 Ultra; Shimadzu, Kyoto, Japan) system. Injector and detector temperatures were set at 260 and 280 ºC respectively. One micro-liter sample was injected and analyzed with the column held initially at 50 ºC for 1

min and then increased up to 280 ºC. The oven temperature programmed to increase from 50 ºC for 3 min to 250 ºC for 2 min and then 280 ºC. It was held at this temperature for 23 ºC. The mass spectra were taken at 71.5 eV with scan interval 0.20 second and fragment from 40 to 650 Da. The total GC run time was 40 min. For GC-MS detection an electron ionization system with ionizing energy 71.5 eV and helium carrier gas at constant flow rate 1.25 ml/min was performed. The identification of the different compounds was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds using National Institute of Standards and Technology (NIST) and Wiley 8 library database.

Statistical analysis

Each experiment was executed in triplicate and results were reported as mean value standard error (SE). Statistical evaluations involve analysis of variance (ANOVA) and Duncan's multiple range tests by using SPSS V.16.0 software. The significant difference at $P \leq 0.05$ from each other was considered as statistically significance. The linear regression analysis was followed for estimation of half maximal effective concentration (EC_{50}) value.

RESULTS AND DISCUSSIONS

Extraction yield and qualitative analysis

The percent extraction yield (w/w) was calculated and it was 30.95% in EE, 30.59 % in EAE and 26.93 % in AQE respectively (Table I). Qualitative analysis of phytoconstituents revealed that phenol, flavonoid, tannin, triterpene, cumarine, anthraquinone and glycosides were present in all extracts (Table II).

TABLE I - Extraction yield from different seed extracts

Extract	Extract weight (g)	Percent extraction yield
EE	6.22	30.95
EAE	6.12	30.59
AQE	5.42	26.93

TABLE II - Qualitative phytochemical constituents of different seed extracts

Test	EE	EAE	AQE
Alkaloid	-	-	_
Phenol	+	+	+
Flavonoid	+	+	+
Tanin	+	+	+
Triterpene and steroid	+	+	+
Cumarine	+	+	++
Saponine	+	+	+
Phlobatanine	-	-	-
Anthraquinone	+	+	++
Glycoside	+	+	+
Protein	-	-	-
+Present/- Absent			

Assay of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The antioxidant activity of seed extracts of *N. arbor-tristis* was evaluated by their ability to scavenge DPPH free radical. All three extracts of seed have significant DPPH free radical scavenging activity (Figure 1). Among these, EAE showed dose dependent inhibition of DPPH activity and scavenging activity of extract increased with increasing concentration. At higher concentration (600µg/ml) extract gave the highest percentage (80.23±0.15) of inhibition of DPPH activity (Figure 1). EAE exhibits comparatively more effective (EC_{50}) total antioxidant capacity (129.43±3.55 µg/ml) than EE (144.47±1.21µg/ml) and AQE (218.67±3.21µg/ml). The EE and AQE exhibited weaker DPPH free radical scavenging activity than EAE. The percentage mean value of half maximal effective concentration (EC_{50}) of reference control (ascorbic acid) to scavenge free radical is 78.72±11.19 µg/ml. The free radical scavenging activity by DPPH was extensively studied to determine the antioxidant capacity of the polyphenol constituents (Mishra *et al.*, 2019). DPPH is nitrogen centered stable free radical and reduced by free electron and hydrogen radical donation and forms a stable diamagnetic molecule (Soares *et al.*, 1997). The color

of DPPH solution was changed from purple to yellow which can be monitored as decrease the absorbance at 517 nm (Canadanovic-Brunet *et al.*, 2014). A methanolic solution of DPPH radicals was converted into $DPPH_2$ (diphenylhydrazine) molecules when mixed with an antioxidant compound that can transfer a hydrogen atom or an electron and is converted into reduced form. All the extracts of seed have shown steady increase in the percent inhibition of $DPPH^{\cdot}$ with increasing concentration. The ability of free radical scavenging activity of seed extracts on the basis of EC_{50} value for DPPH free radical was as follows: EAE > EE > AQE. Our results is in tuned with another investigation that ethyl acetate extract of seed of *N. arbor-tristis* was very potent and exhibited significant DPPH free radical scavenging activity (IC_{50} 459.91±1.40 µg/ml) (Vajravijayan *et al.*, 2013). In another study ethyl acetate extract of leaf of *N. arbor-tristis* showed noticeable percentage of DPPH free radical scavenging effect (69.68%) at 500 µg/ml, while other extracts showed lesser DPPH scavenging effect (Karan *et al.*, 2019). These results evidently supports that EAE has potential antioxidant activity.

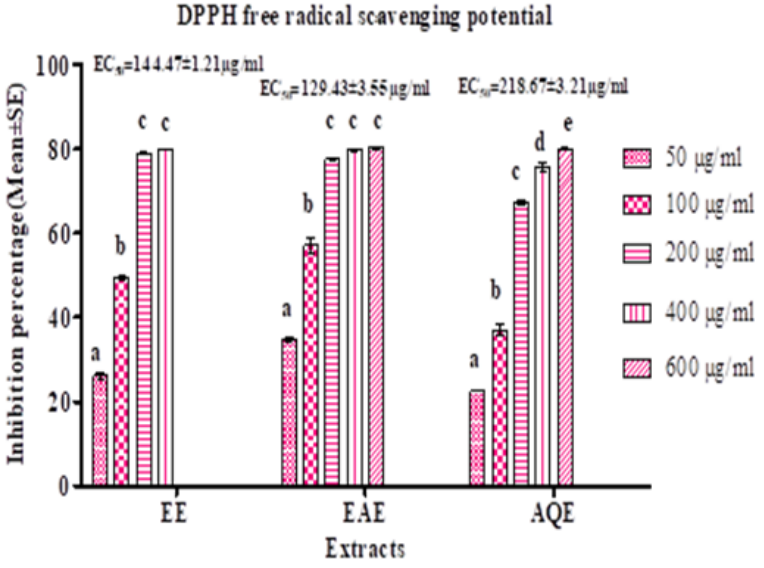


FIGURE 1 - DPPH free radical scavenging potential in different seed extracts of *N. arbor-tristis* L. Different letters indicate the significant differences from each other at $P \leq 0.05$.

Assay of superoxide anion free radical scavenging activity

Extracts of seeds have potent scavenging activity for superoxide radicals such as H_2O_2, $OH^{\cdot}$ and $^1O_2$. The results of the superoxide radical scavenging activity of all three extracts were as follows: EAE (969.94±8.03µg/ml) followed by EE (1087.33±6.22 µg/ml) and AQE (1214.83±9.65 µg/ml). Overall, EAE was comparatively more potent for free radical scavenging activity than other two extracts (Figure 2). The percentage mean value (EC_{50}) of standard (copper sulphate) was 337.82±0.18 µg/ml. Superoxide anions radical is a harmful species to cellular components and are produced by a number of cellular reactions including xanthine oxidase, lipooxygenases, NADPH oxidases and peroxidases. They are precursor of reactive oxygen species and induced damages to biomolecules including lipid, protein and DNA (Mandade, Sreenivas, Choudhury, 2011). The conversion of superoxide and H_2O_2 into more reactive species, like hydroxyl radical ($OH^{\cdot}$) is one of the unfavorable effect caused by superoxide radicals (Hazra, Biswas, Mandal, 2008). The radical scavenging activity is usually related to the presence of hydroxyl group in aromatic rings of

the phenolic compounds (Brand-Williams, Cuvelier and Berset, 1995). Superoxide anion reduces the yellow dye (NBT^{2+}) to generate the blue intensity formazan, which is determined spectrophotometrically at 560 nm. In present study, all extracts of seeds are found to be an effective scavenger of singlet oxygen generated in riboflavin-NBT-light system. It revealed that the scavenging activity of all extracts was correlated with increase in the concentration of extracts. The inhibition of NBT blue colour complex formation is the result of antioxidant activity (Rahman, Imran, Islam, 2013). In this assay the EAE has greater scavenging potential than EE and AQE.

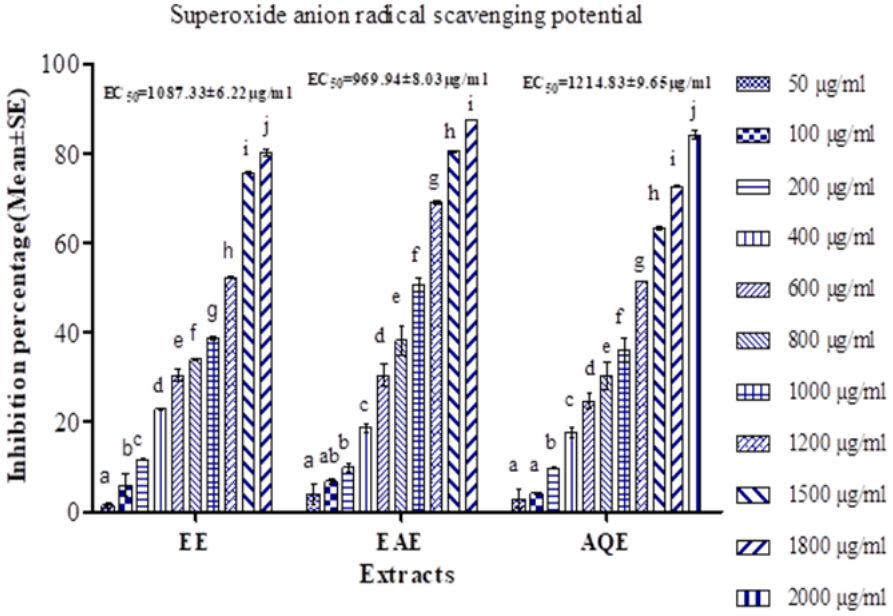


FIGURE 2 - Superoxide anion free radical scavenging potential of *N. arbor-tristis* L. seed extracts. Different letters indicate the significant differences from each other at P ≤0.05.

Assay of ferric reducing antioxidant power (FRAP)

FRAP of different extracts was increased with increased concentration of the extracts. It was found that higher absorbance at 700 nm indicates higher reducing power. The reducing power activities of all three extracts at 1000 µg/ml were decreased in this order: EAE ($A_{700\ nm}$ = 0.245±0.014) > EE ($A_{700\ nm}$ = 0.220±0.010) > AQE ($A_{700\ nm}$ = 0.201±0.010) (Figure 3). The same concentration (1000 µg/ml) of ascorbic acid which is used as a positive control in this assay had ferric reducing antioxidant power value of 0.645±0.031 at 700 nm. The ferric reducing power of the extracts was linearly proportional to the concentration of the sample. The increased absorbance of reaction mixture indicates the stronger reducing power. FRAP assay used for the study of antioxidant activity was based on the change in the color of the test solution from yellow to various shades of green, depending on the reducing power of the extract. The degree of color change was correlated with the sample's antioxidant activity. In this assay during reaction the reduction of Fe (III) to Fe (II) took place by antioxidants and form perl's purssian blue (Kim *et al*., 2014). Reducer concentrations directly linked with Fe^{2+} concentration which can be monitored by the absorbance of samples. These reducers showed their antioxidant action by breaking the free radical chain by donating a hydrogen atom and also react with certain precursors of peroxide. In this study, FRAP of all extracts was increased with increasing its concentration. Generally, the reducing power activities was based on the breakdown of the free radical chain by donating a hydrogen atom and also reaction with the other precursor

of peroxide, which in turn prevent the peroxide formation, as absorbing and neutralizing free radicals, quenching singlet and triplet oxygen (Yildirim *et al.*, 2000). It was also observed a direct correlation between antioxidant activity and reducing power of plant extracts (Ferreira *et al.*, 2007). The reducing capacity of extracts may serve as a significant indicator of its potential antioxidant activity. Our results shows that EAE is an electron donor and could react with free radicals and convert them to more stable product and also terminate the radical chain reaction. At the higher concentration (1000 µg/ml) the ferric reducing antioxidant power of EAE of seeds of *N. arbor- tristis* and positive control ascorbic acid was 0.245±0.014 and 0.645±0.031 respectively.

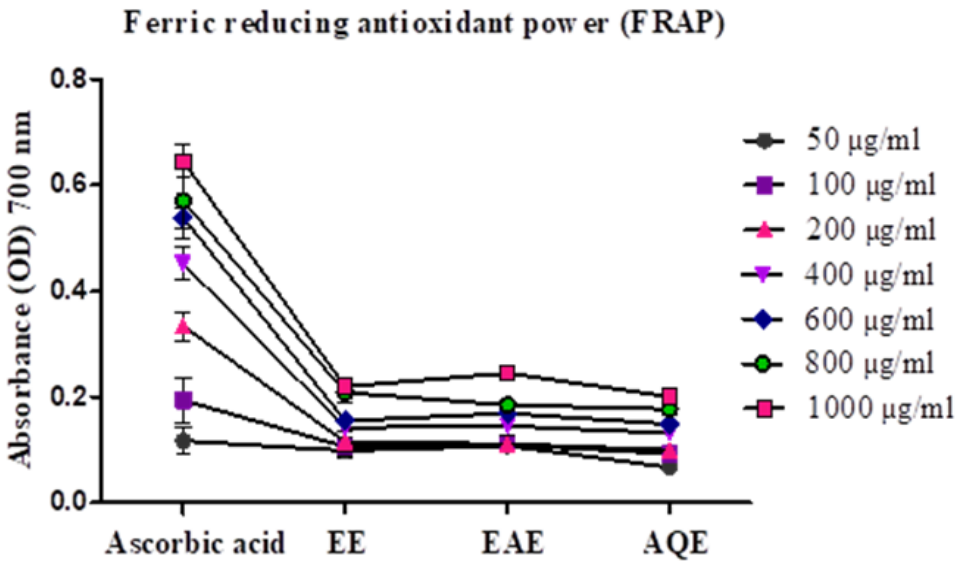


FIGURE 3 - Ferric reducing antioxidant power (FRAP) of ascorbic acids and different seed extracts (EE, EAE and AQE) of *N. arbor-tristis* L.

Determination of lipid peroxidation inhibition potential (LPIP)

The extracts of seeds have excellent LPIP (Figure 4). The EAE was more effective for lipid peroxidation inhibition potential (EC_{50} 452.43±5.07 µg/ml) than other extracts. The trend of LPIP in all three extracts was in this order: EAE > EE > AQE (Figure 4). The effective mean value of half maximal effective concentration (EC_{50}) of reference compound (ascorbic acid) for lipid peroxidation inhibition potential was 297.67±0.39 µg/ml. The oxidative damage may lead to the lipid peroxide formation. In lipid peroxidation, oxidative deterioration of polyunsaturated fatty acids (PUFA) either in the form of free fatty acids (FFAs) or triacylglycerides (TAGs) was took place. In general, membrane phospholipids (mostly glycolipids, phospholipids, and sphingolipids) and cholesterol are the major targets of the oxidative damage in biological systems. It is due to the presence of methylene groups adjacent to double bonds. The lipid peroxidation caused oxidative cleavage of PUFA, which induced cell injury and leading to formation of malondialdehyde (MDA) (Ayala, Munoz, Arguelles, 2014). The initiation of peroxidation reaction in membrane or PUFA is the result of abstraction of hydrogen atom from double bond in fatty acids. The free radical, initiated oxidative chain reaction by molecular rearrangement and reaction with methylene group of PUFA to produce a conjugated dienes, which reacts with an oxygen molecule to produce a peroxy radical (Blokhina, Virolainen, Fagerstedt, 2003). Peroxy radical is highly reactive to propagate the chain reaction of lipid peroxidation to form cyclic peroxidase. During the formation of cyclic peroxidase, lipid molecules gradually oxidized to the maximum possible extent. In

biological system lipid peroxidation generates a number of degradation products such as MDA. MDA is a cytotoxic product of PUFA peroxidation, which is highly reactive and toxic. It interacts with proteins and DNA and caused loss of protein function and DNA mutations. MDA accumulation is a key sign of the occurrence of lipid peroxidation and has been widely used as the key indicator of oxidative stress. In general, peroxidation of lipid bilayer membrane further assisted in the creation of pores, which may help in the penetration of reactive oxygen species, and eventually causing the oxidative stress.

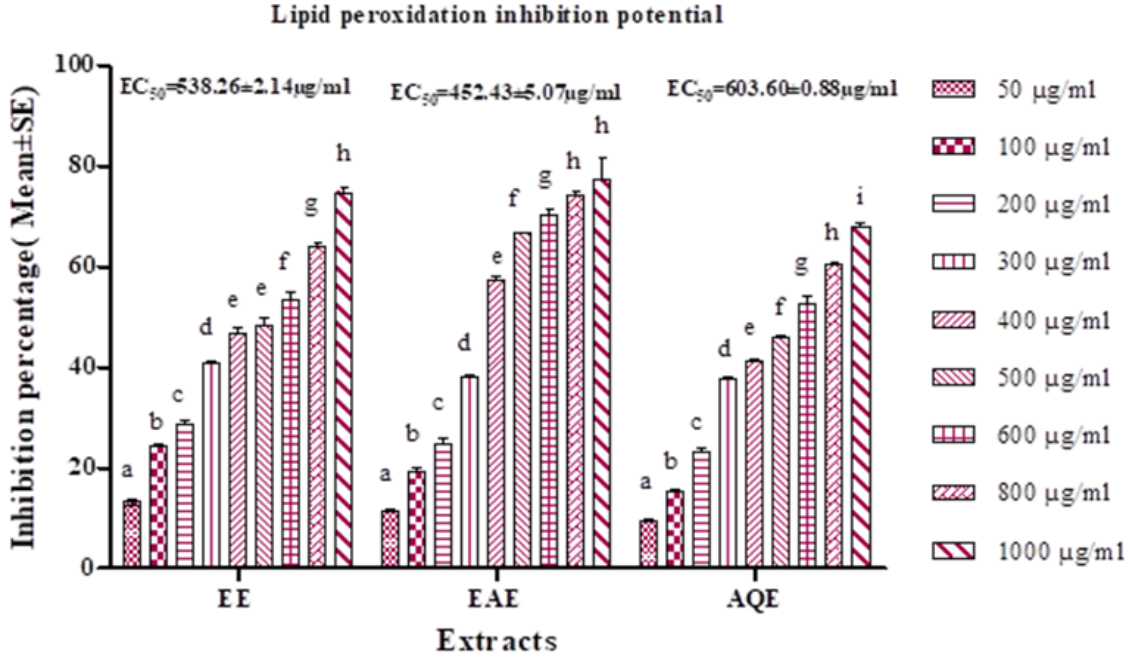


FIGURE 4 - Lipid peroxidation inhibition potential (LPIP) of seed extracts. Different letters indicate the significant differences from each other at $P \leq 0.05$.

Total phenolic content (TPC) and Total flavonoid Content (TFC)

TPC was determined by the Folin-Ciocalteu (FC) method. GAE was used as standard to make standard curve. The amount of TPC was highest in AQE (201.00±0.20 µg/mg GAE). The amount of phenol in EAE and EE was 159.50±0.28 µg/mg and 187.50 ± 0.25µg/mg respectively. The amount of phenol content in EAE was comparatively quit low than other two extracts of the seeds (Figure 5). It revealed that extraction in high polar solvent was more effective for TPC. Phenol and flavonoid are secondary metabolites present in the plants acts as free radical scavengers and play vital role in protection from adverse conditions such as UV radiation, injury and infection (Ayyanar, Subash-babu, 2012). These compounds were formed in seeds during its growth and maturity (Zhang, Li, Cheng, 2010). The phenolic compounds act as antioxidant and significantly involved in the free radical scavenging, oxygen radical absorbance, and chelation of transition metal ions (Hossain *et al*., 2016). The marked effect of phenolic is the prevention of oxidative stress induced diseases. Results revealed that phenolic content was greater in AQE than EE and EAE. Phenols act as a good antioxidant and these properties of the phenol was directly linked to their structure. Due to the presence of multiple aromatic rings bearing hydroxyl groups are capable to quench the colour of stable free radical (Bozin *et al*., 2008). The amount of total flavonoid content (TFC) was determined by aluminium chloride ($AlCl_3$)

colorimetric method and rutin was used as standard for making standard curve. The total flavonoid content in the *N. arbor-tristis* seed extracts was measured and it was found that TFC was maximum in the EAE (34.50±0.40 µg/mg RE) in comparison to EE (27.12±0.18 µg/mg) and AQE (20.62±0.25 µg/mg RE) respectively (Figure 5). Flavonoids are also well-known antioxidant and are present in plants, containing a number of hydroxyl groups attached with ring structure and possess a broad spectrum chemical and biological activities (Nimse, Pal, 2015). TFC in the extracts was analyzed and it was noted that flavonoid content was greater in EAE than EE and AQE.

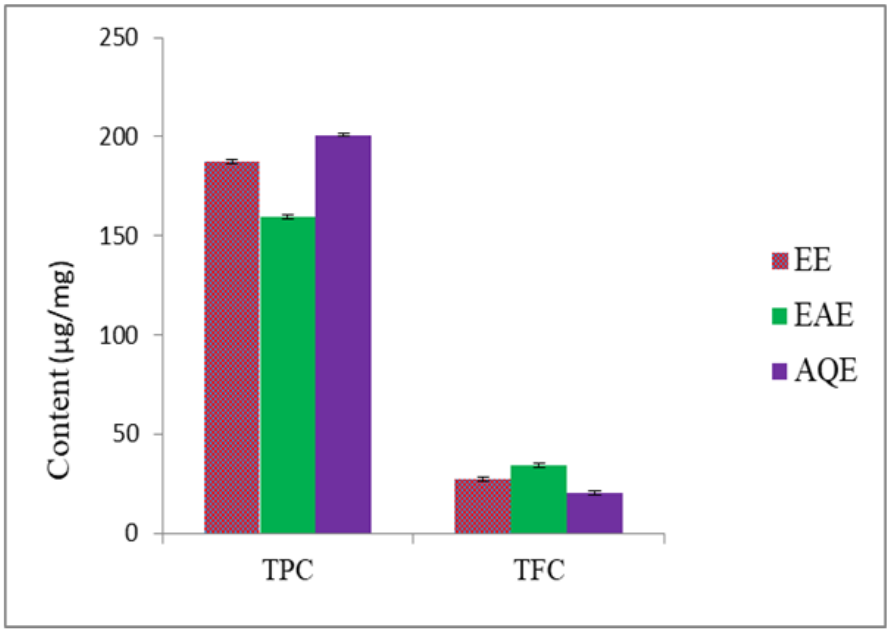


FIGURE 5 - Polyphenol content (TPC and TFC) in different extracts of seed of *N. arbor-tristis* L.

Correlation matrix between total phenol, flavonoid and antioxidant activity

Correlation between TPC, TFC and antioxidant activity was measured using Pearson's correlation test. Correlation matrix of DPPH, FRAP, SO radical, LPIP, TPC and TFC was shown in Table III. A significant, strong positive correlation was found between TPC and LPIP (0.993), superoxide anion radical (SO radical) (0.975) and DPPH free radical scavenging activity (0.845). Similarly, a significant positive correlation ($P \leq 0.05$) was found between TFC and FRAP (0.999), while a significant negative correlation with superoxide anion radical (SO radical) scavenging activity (-0.998) and LPIP (-0.999) was recorded. The positive correlation (0.845) was found between the total phenol and DPPH assay. The strong negative correlation (-1.000) ($P \leq 0.01$) was found between LPIP and FRAP. These results indicate that there was strong relationship between phenolic components in seed extracts with antioxidant activity. In conclusion, we can say that the presence of flavonoid and phenol constituents in the seed extracts are significantly responsible for antioxidant potential.

TABLE III - Correlation matrix between antioxidant potential, total phenol and flavonoid content

	DPPH	FRAP	SO Radical	LPIP	TPC	TFC
DPPH	1					
FRAP	-0.903	1				
SO Radical	0.942	-0.995	1			
LPIP	0.903	-1.000**	0.995	1		
TPC	0.845	-0.993	0.975	0.993	1	
TFC	-0.920	0.999*	-0.998*	-0.999*	-0.987	1

** Correlation is significant at the $P \leq 0.01$ level.
*Correlation is significant at the $P \leq 0.05$ level.

Gas chromatography mass spectrometry (GC-MS) profile of EAE of seed

The interpretation of mass spectrum was conducted using database of National Institute of Standards and Technology (NIST) and Wiley 8 library. Gas chromatography mass spectrometry (GC–MS) chromatogram of EAE (Figure 6) shows the retention time and detected peaks which correspond to the bioactive compounds present in the extract. The GC-MS profile showed the presence of 29 different phytochemical compounds. The relative quantity of the chemical compounds present in the extract was expressed as percentage (%) based on peak area. The retention time, peak area percentage (%), chemical structures of the identified compounds with their molecular weights are summarized in Table IV. In gas chromatograph mass spectrometry analysis of EAE showed retention time ranged from 5.65 to 40.00 (Figure 6). Based on the abundance the most prevailing identified chemical compounds with retention time and high peak area percent in EAE were as follows: 1,2,3-propanetriol,1-acetate (RT 5.650, 1.63%), pentadecanoic acid (RT 16.632,9.46%), 9-octadecanoic acid methyl ester (RT 17.816,1.41%), octadec-9-enoic acid (RT 18.520,72.83%), humulane-1,6-dien-3-ol (RT 28.389, 1.16%), tetracontane (RT 35.347, 1.53%), and olean-12-en-3one (RT 35.563, 1.84%) respectively. The GC-MS analysis of EAE revealed the presence of two major compounds with maximum peak area percent namely pentadecanoic acid (9.46%) and Octadec-9-enoic acid (72.83%). The other studies revealed that the identified compounds possess various pharmacological activities. The 1,2,3-Propanetriol, 1- acetate present in the extract (RT 5.650) exhibit antifungal, anticancer, anti-inflammatory potential (Foo, Salleh, Mamat, 2015). 1,2,3-Propanetriol, 1- acetate was proven to be a precursor of tricetin (antifungal), but may also serve as a pro-drug and vehicle for anticancer agents (Juneious, 2014). The most prevailing component in the extract, pentadecanoic acid (RT 16.632) possesses antioxidant activity (Vijisaral Elezabeth, Arumugam, 2014). 9-octadecanoic acid methyl ester (RT 17.816) is reported to have antifungal and antibacterial properties (Arora, Kumar, Meena, 2017). The compound octadec-9-enoic acid (RT 18.520) possesses antihypertensive properties (Arora, Kumar, 2018). Humulane-1, 6-dien-3-ol (RT 28.389) has hypo-cholesterolemic activity (Arora, Kumar, 2018). Tetracontane (RT 35.347) has anti-inflammatory activity (Arora, Meena, 2018).The biological activities of the compounds present in the EAE of the seeds support the medicinal value of the seeds. However, further studies will be needed to isolate the novel phytocomponents of *N. arbor-tristis* L. seed and to investigate their biological activities.

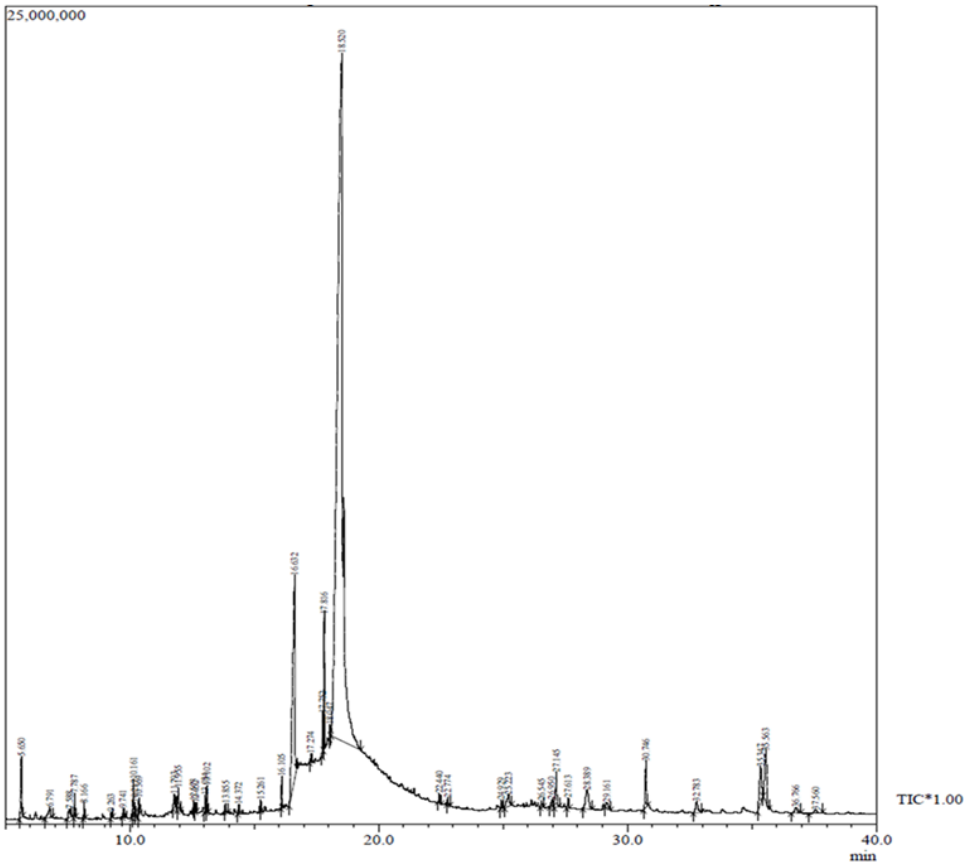


FIGURE 6 - Gas chromatography mass spectrometry (GC-MS) profile EAE of seed of *N. arbor-tristis* L.

TABLE IV - List of phytocomponents identified by Gas Chromatography Mass Spectrometry (GC-MS) analysis of EAE of *N. arbor-tristis* L. seed and their bioactivity

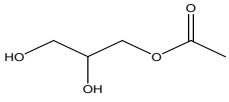
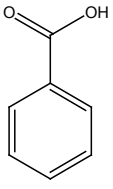
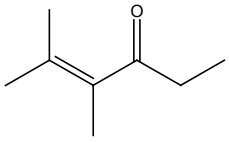
S. No.	Retention time (min)	Compound identified	Structure	Molecular formula	Molecular weight	Peak area %	Reported activity	Reference
1	5.650	1,2,3-Propanetriol, 1- acetate		$C_5H_{10}O_4$	134	1.63	Anti-fungal and anticancer, anti-inflammatory	Foo, Salleh, Mamat, 2015
2	6.791	Benzoic acid		$C_7H_6O_2$	122	0.42	Antimicrobial	Foo, Salleh, Mamat, 2015
3	7.588	4-Hexen-3-one,4,5-dimethyl		$C_8H_{14}O$	126	0.31	No activity is reported	_____

TABLE IV - List of phytocomponents identified by Gas Chromatography Mass Spectrometry (GC-MS) analysis of EAE of *N. arbortristis* L. seed and their bioactivity

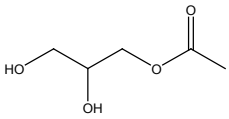
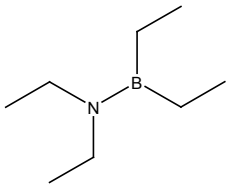
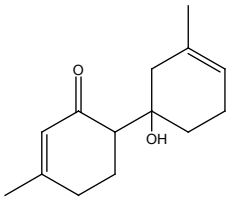
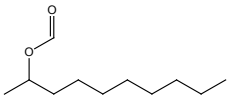
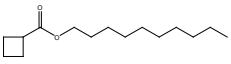
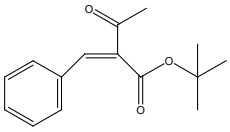
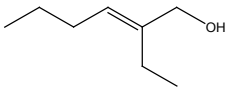
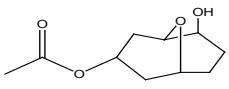
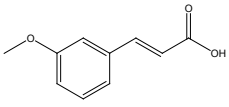
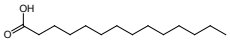
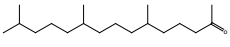
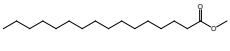
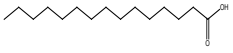
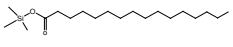
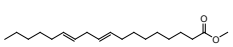
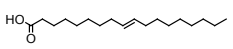
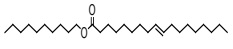
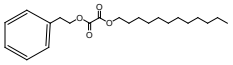
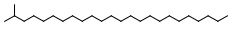
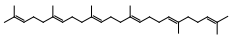
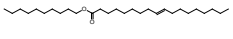
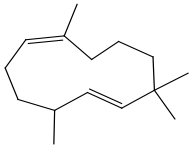
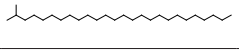
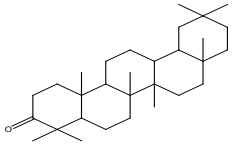
S. No.	Retention time (min)	Compound identified	Structure	Molecular formula	Molecular weight	Peak area %	Reported activity	Reference
4	7.787	2,3-Dihydroxypropyl acetate		$C_5H_{10}O_4$	134	0.34	No activity is reported	_____
5	9.263	Boranamine, N,N,1,1-Tetraethyl		$C_8H_{20}BN$	141	0.08	No activity is reported	_____
6	10.161	1- Hydroxy-4,3-dimethyl-bicyclohexyl-3,3-dien-2-one		$C_{14}H_{20}O_2$	220	0.59	No activity is reported	_____
7	10.217	Formic acid,dec-2-yl ester		$C_{11}H_{22}O_2$	186	0.14	No activity is reported	_____
8	11.955	Cyclobutanecarboxylic acid, decyl ester		$C_{15}H_{28}O_2$	240	0.45	No activity is reported	_____
9	12.573	2-Acetyl-3-Phenyl-Acrylic Acid Tert-Butyl Ester		$C_{15}H_{18}O_3$	246	0.11	No activity is reported	_____
10	12.652	2-Hexen-1-ol, 2 Ethyl		$C_8H_{16}O$	128	0.11	No activity is reported	_____
11	13.034	8-Oxabicyclo[3.2.1] octan-3,7-diol,3-acetate		$C_{10}H_{16}O_4$	200	0.20	No activity is reported	_____
12	13.855	2-PropenoicAcid, 3-(4-Methoxyphenyl)		$C_{10}H_{10}O_3$	178	0.18	No activity is reported	_____
13	14.372	Tetradecanoic acid		$C_{14}H_{28}O_2$	228	0.10	Antioxidant	Krishnamoorthy, Subramaniam, 2014
14	15.261	2-Pentadecanone,6,10,14-trimethyl		$C_{18}H_{36}O$	268	0.11	Anti-bacterial	Arora, Kumar, Meena, 2017
15	16.105	Hexadecanoic Acid, Methyl Ester		$C_{17}H_{34}O_2$	270	0.31	Anti-inflammatory, antiarthritic, nematicide, antihistaminic and cancer preventive; Antimicrobial	Krishnamoorthy, Subramaniam, 2014; Rahuman *et al.*, 2000

TABLE IV - List of phytocomponents identified by Gas Chromatography Mass Spectrometry (GC-MS) analysis of EAE of *N. arbor-tristis* L. seed and their bioactivity

S. No.	Retention time (min)	Compound identified	Structure	Molecular formula	Molecular weight	Peak area %	Reported activity	Reference
16	16.632	Pentadecanoic acid		$C_{15}H_{30}O_2$	242	9.46	Antioxidant	Vijisaral Elezabeth, Arumugam, 2014
17	17.274	Hexadecanoic acid, Trimethylsilyl Ester		$C_{19}H_{40}O_2Si$	328	0.14	No activity reported	_____
18	17.752	9,12-Octadecadienoic acid, methyl ester		$C_{19}H_{34}O_2$	294	0.42	Anti-inflammatory, antiarthritic, nematicide, antihistaminic and cancer preventive	Krishnamoorthy, Subramaniam, 2014
19	17.816	9-Octadecanoic acid methyl ester		$C_{19}H_{36}O_2$	296	1.41	Anti-fungal, Anti-bacterial,	Arora, Kumar, Meena, 2017
20	18.520	Octadec-9-enoic acid		$C_{18}H_{34}O_2$	282	72.83	Antihypertensive	Arora, Kumar, 2018
21	22.774	Decyl oleate		$C_{28}H_{54}O_2$	422	0.13	Cancer-preventive, Hypocholesterolemic, Perfumery, Anti-inflammatory, Dermatitigenic,	Ponmathi *et al*., 2017
22	24.929	Oxalic acid, dodecyl 2-phenylethyl ester		$C_{22}H_{34}O_4$	362	0.15	No activity is reported	_____
23	25.223	2-Methyltetracosane		$C_{25}H_{52}$	352	0.61	Free radical scavenging	Arora, Kumar, Meena, 2017
24	26.545	Squalene		$C_{30}H_{50}$	410	0.09	Antioxidant, anti-inflammatory, and anti-atherosclerotic properties	Lou-Bonafonte *et al*., 2018
25	26.950	9-Octadecenoic acid(Z)-,Tetradecyl ester		$C_{28}H_{54}O_2$	534	0.19	No activity is reported	_____
26	28.389	Humulane-1,6-dien-3-ol		$C_{15}H_{26}O$	222	1.16	Hypocholesterolemic	Arora, Kumar, 2018
27	32.783	2-methylhexacosane		$C_{27}H_{56}$	380	0.51	Antimirobial, Decreases Blood Cholesterol	Arora, Kumar, 2018
28	35.347	Tetracontane		$C_{40}H_{82}$	562	1.53	Anti-inflammatory	Arora, Meena, 2018
29	35.563	Olean-12-en-3 one		$C_{30}H_{48}O$	424	1.84	No activity is reported	_____

CONCLUSION

On the basis of above study, it may be concluded that EAE was superior to EE and AQE for antioxidant activity. EAE was rich in polyphenols which may be responsible for antioxidant activity. In addition, there was a good correlation between phenolic content and antioxidant capacity of the seed extracts. The results confirmed that seeds of the *N. arbor-tristis* L. possess effective antioxidant properties. The presence of various bioactive compounds in EAE was confirmed by GC-MS analysis. Presence of different phytoconstituents with various biological activities supports its medicinal applications and can be recommended for the pharmacological applications of seeds. However, further, study is needed to isolate and purify the novel active compounds for various pharmacological activities, which may be useful in the treatment of various health complications.

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REFERENCES

Adjimani JP, Asare P. Antioxidant and free radical scavenging activity of iron chelators. Toxicol Rep. 2015;2:721-28.

Apak R, Ozyurek M, Guclu K, Capanoglu E. Antioxidant activity/capacity measurement reactive oxygen and nitrogen species (ROS/RNS) scavenging assays, oxidative stress biomarkers, and chromatographic/chemometric assays. J Agri Food Chem. 2016;64(5):1046-70. DOI: 10.1021/acs.jafc.5b04744.

Arora S, Kumar G, Meena S. Screening and evaluation of bioactive components of *Cenchrus ciliaris* (L.) by GCMS analysis. Int Res J Pharm. 2017;8(6):69-76.

Arora S, Kumar G. Phytochemical screening of root, stem and leaves of *Cenchrus biflorus* Roxb. J Pharmacogn Phytochem. 2018;7(1):1445-1450.

Arora S, Meena S. Analysis of bioactive constituents from *Ceropegia bulbosa* Roxb. Var. *Bulbosa*: An endangered medicinal plant from Thar Desert of Rajasthan, India. J Pharmacog Phytochem. 2018;7(1):2242-47.

Ayala A, Munoz MF, Arguelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014:1-31. DOI.org/10.1155/2014/360438.

Ayyanar M, Subash-babu P. *Syzygium cumini* (L.) Skeels: a review of its phytochemical constituents and traditional uses. Asian Paci J Trop Biomed. 2012;2(3):240-46.

Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971;44(1):276-87.

Bhandary SK, Kumari NS, Bhat VS, Sharmila KP, Bekal MP. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruits and seeds. Nitte Univ J Health Sci. (NUJHS). 2012;2(4):34-8.

Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot. 2003;91Spec No(2):179-94.

Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Igic R. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). Food Chem. 2008;111(4):925-29.

Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate anti-oxidant activity. LWT- Food Sci Technol. 1995;28(1):25-30.

Canadanovic Brunet J, Cetkovic G, Saponjac VT, Stajcic S, Vulic J, Djilas S, et al. Evaluation of phenolic content, antioxidant activity and sensory characteristics of Serbian honey- based product. Ind Crops Prod. 2014;62:1-7.

Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in *Propolis* by two complementary colometric methods. J Food Drug Anal. 2002;10(3):178-82.

Das S, Sasmal D, Basu SP. Anti-inflammatory and antinociceptive activity of arbor-tristosides A. J Ethnopharmcol. 2008;116(1):198-203.

Das S, Sasmal D, Basu SP. Antispasmodic and anthelmintic activity of *Nyctanthes arbortristis* Linn. Int J Pharm Sci Res. 2010;1(2):51-55.

Dzoyem JP, Eloff JN. Anti-inflammatory, anticholinesterase and antioxidant activity of leaf extract of twelve plants used traditionally to alleviate pain and inflammation in South Africa. J Ethanopharmacol. 2015;160:194-201.

Ferreira ICFR, Baptista P, Vilas-Boas M, Barros L. Free-radical scavenging capacity and reducing power of wild

edible mushrooms from northeast Portugal: individual cap and stipe activity. Food Chem. 2007;100(4):1511-16.

Foo LW, Salleh E, Mamat SNH. Extraction and qualitative analysis of *Piper betle* leaves for antimicrobial activities. Int J Eng Technol Sci Res. 2015;2Special Issue:1-8.

Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* Indigenous to Balochistan. Sci World J. 2017;2017:1-7. DOI:10.1155/2017/5873648.

Gupta P, Bajpai SK, Chandra K, Singh KL, Tandon JS. Anti-viral profile of *Nyctanthes arbor-tristis* L. against encephalitis causing viruses. Indian J Exp Biol. 2005;43(12):1156-60.

Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity *Spondias pinnata*. BMC Complement Alt Med. 2008;8:63. DOI:10.1186/1472-6882-8-63.

Hossain H, Rahman SE, Akbar PN, Khan TA, Rahman M, Jahan IR. HPLC profiling, anti-oxidant and *in vivo* anti-inflammatory activity of the ethanol extract of *Syzygium jambos* available in Bangladesh. BMC Res Notes. 2016;9:191-98.

Juneious CE. Molecular biological determination of PKC inhibitory effects of 1, 2, 3-propanetriol monoacetate produced form marine sponge associated bacteria. In: 3rd International Conference on Clinical Microbioloby & Microbial Genomics; 2014 Sep 24–26; Valencia, Spain.

Karan BN, Maity TK, Pal BC, Singha T, Jana S. Betulinic Acid, the first lupane-type triterpenoid isolated via bioactivity-guided fractionation, and identified by spectroscopic analysis from leaves of *Nyctanthes arbor-tristis*: its potential biological activities in vitro assays. Nat Prod Res. 2019;33(22):3287-92. DOI:10.1080/14786419.2018.1470171.

Karmakar I, Dolai N, Saha P, Sarkar N, Bala A, Haldar PK. Scavenging activity of *Curcuma caesia* rhizome against reactive oxygen and nitrogen species. Orient Pharm Exp Med. 2011;11(4):221-28.

Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci. 2015;11(8):982-91.

Kim SJ, Matsushita Y, Fukushima K, Aoki D, Yagami S, Yuk HG, Lee SC. Antioxidant activity of a hydrothermal extract from watermelons. LWT Food Sci Technol. 2014;59(1):361-68.

Krishnamoorthy K, Subramaniam P. Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi using GC-MS. Int Sch Res Notices. 2014;2014:1-13. http://dx.doi.org/10.1155/2014/567409.

Kumari P, Sahal D, Jain SK, Chauhan VS. Bioactivity guided fractionation of leaves extract of *Nyctanthes arbor-tristis* (Harshringar) against *P. falciparum*. PLoS ONE. 2012;7(12):e51714. Doi.org/10.1371/journal.pone.0051714.

Li S, Chen G, Zhang C, Wu M, Wu S, Liu Q. Research progress of natural anti-oxidants in food for the treatment of diseases. Food Sci Human Well J. 2014;3(3-4):110-16.

Liu J, Jia L, Kan J, Jin CH. *In vitro* and *in vivo* antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). Food Chem Toxicol. 2013;51:310-316.

Lou-Bonafonte MJ, Martinez-Beamonte R, Sanclemente T, Surra JC, Herrera-Marcos LV, Sanchez-Marco J, et al. Current insights into the biological action of squalene. Mol Nutr Food Res. 2018;62(15):e1800136. https://doi.org/10.1002/mnfr.201800136.

Mandade R, Sreenivas SA, Choudhury A. Radical scavenging and anti-oxidant activity of *Carthamus tinctorius* extracts. Free Rad Antioxid. 2011;1(3):87-93.

Maria R, Shirley M, Xavier C, Jaime S, David V, Rosa S, Jodie D. Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. J King Saud Univ Sci. 2018;30(4):500-505. doi.org/10.1016/j.jksus.2017.03.009.

Mc Donald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem. 2001;73(1):73-84.

Mishra AK, Tiwari KN, Mishra P, Tiwari SK, Mishra SK, Saini R. Effect of cytokinin and MS medium composition on efficient shoot proliferation of *Nyctanthes arbor-tristis* L. through cotyledonary node explant and evaluation of genetic fidelity and antioxidant capacity of regenerants. South Afr J Bot. 2019;127:284-92.

Mishra AK, Upadhyay R, Chaurasia JK, Tiwari KN. Comparative antioxidant study in different flower extracts of *Nyctanthes arbor-tristis* L. (Oleaceae): an important medicinal plant. Braz J Bot. 2016;39(3):813-20.

Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. R Soc Chem Adv. 2015;5:27986-28006. DOI:10.1039/c4ra13315c.

Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-58.

Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese J Nutr Diet. 1986;44(6):307-315.

Pandey MM, Rastogi S, Rawat AKS. Indian traditional ayurvedic system of medicine and nutritional supplementation. Evid-Based Complement Alternat Med. 2013;2013:1-12. http://dx.doi.org/10.1155/2013/376327.

Ponmathi SA, Michael ER, Muthukumarasamy S, Mohan VR. Determination of bioactive components of *Barleria courtallica* nees (acanthaceae) by gas chromatography–mass spectrometry analysis. Asian J Pharm Clin Res. 2017;10(6):273-83.

Rahman MA, Imran TB, Islam S. Anti-oxidative, antimicrobial and cytotoxic effects of the phenolics of *Leea indica* leaf extract. Saudi J Biol Sci. 2013;20(3):213-25.

Rahman MM, Roy SK, Shahjahan M. Fatty acid composition of ripe seed oil of *Nyctanthes arbor-tristis* L. J Bangladesh Acad Sci. 2011;35(1):121-24.

Rahuman AA, Gopalakrishnan G, Ghouse BS, Arumugam S, Himalayan B. Effect of *Feronia limonia* on mosquito larvae. Fitoterapia. 2000;71:553-55.

Sharma L, Dhiman M, Singh A, Sharma MM. *Nyctanthes arbor-tristis* L.: an unexplored plant of enormous possibilities for economic revenue. Proc Natl Acad Sci India Sect B. 2021;91(2):241-255. https://doi.org/10.1007/s40011-020-01213-y.

Soares JR, Dins TCP, Cunha AP, Almeida LM. Antioxidant activity of some extracts of *Thymus zygis*. Free Rad Res. 1997;26(5):469.

Tandon JS, Srivastava V, Guru PY. Iridoid: a new class of leishmanicidal agent from *Nyctanthes arbor-tristis*. J Nat Prod. 1991;54(4):1102-1104.

Vajravijayan S, Udayakumar M, Brabakaran A, Thangaraju N. Anti–inflammatory, antioxidant and free radical scavenging activities of *Nyctanthes arbor-tristis Linn*. Seed extract under *in vitro*. Sch Acad J Biosci. 2013;1(6):242-50.

Vijisaral Elezabeth D, Arumugam S. GC-MS analysis of bioactive constituents of *Indigofera suffruticosa* leaves. J Chem Pharm Res. 2014;6(8):294-300.

Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V. Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea* Desf Ex DC), Sage (*Savia triloba* L.), and Black tea (*Camellia sinensis*) extracts. J Agri Food Chem. 2000;48(10):5030-34.

Zhang Y, Li P, Cheng L. Developmental changes of carbohydrates, organic acids, amino acids, and phenolic compounds in 'Honeycrisp' apple flesh. Food Chem. 2010;123(4):1013-18.

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