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Screening of antioxidant and free radical scavenging activities of *Terminalia arjuna* Roxb

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Abstract
This present work was carried out to quantify the total phenolic and total flavonoid content and to investigate the antioxidant activity free radical scavenging activities of leaf of extracts of *Terminalia arjuna* (arjuna). Leaf extracts of *Terminalia arjuna* were prepared by Soxhlet extraction and various extracts were used for in-vitro assays. The extraction yields of whereas *Terminalia arjuna* 3-12g/50g (w/w) on dry weight basis The qualitative phytochemical studies revealed presence of alkaloid, saponin, flavonoid, tannin, carbohydrates, glycosides in different extracts of *Terminalia arjuna* leaves. The results indicated the presence of higher phenolic and flavonoid content in aqueous leaf extracts of *Terminalia arjuna*. It was observed that *Terminalia arjuna* contained appreciable amount of TPC (37.696-42.229ugGAE/mg) and TFC (44.686-263.41ugQE/mg) as well as exhibited good DPPH radical scavenging activity (10.47-11.13ug/ml) and nitric oxide radical scavenging activity (9.49-11.32ug/ml). The results of the present investigation clearly demonstrated the significant variations in antioxidant properties of different solvents extract of *Terminalia arjuna* leaves. It can be concluded from results that arjuna extracts were good source of natural antioxidants.

Keywords: Antioxidant, *Terminalia arjuna*, TPC, TFC, IC_{50}, DPPH, NO radical scavenging activity

Introduction
Plants have a long history of usage as therapeutic agents and were the main source of medicines prior to the advances of modern medicine. In many developing countries, herbal medicinal systems remain important in the treatment of many ailments (Kamboj 2000) [1]. Phenolic compounds are generally strong antioxidants. Their primary action involves the protection of cell constituents against oxidative damage through the scavenging of free radicals, herby averting their deleterious effects on nucleic acids, proteins and lipids in cells (Rice-Evans *et al*., 1997) [2]. Although our body possess natural antioxidant defense mechanism to protect oxidative stress but antioxidants from natural sources could provide enhanced protection against diseases. Medicinal plants are rich source of phenolic compounds and have large number of biological effects including antioxidant activity which may help to protect the cells against the oxidative damage caused by free radicals (Mandal *et al*., 2013) [3].
The plant, *Terminalia arjuna* Roxb is commonly known as arjuna belongs to the family of Combretaceae. *Terminalia arjuna* Roxb contains high levels of the antioxidant compounds including glycosides, flavonoids, tannins and inorganic minerals. Its bark powder possesses asthma relieving, diuretic, prostaglandin enhancing and coronary risk factor modulating properties (Tripathi 1996, Udupa 1986) [4, 5].
Therefore the present study was undertaken to evaluate phenolic and flavonoids content and antioxidant activity in different extracts of leaf of *Terminalia arjuna* to study the beneficial effects in treatments of different disorders.

Materials and Methods
Different chemicals 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3-(2- pyridyl)- 5,6-diphenyl-12,4-triazine-4 , 4disulfonic acid, potassium ferricyanide, Quercetin, Gallic acid, ascorbic acid, Folin –Ciocalteu phenol reagent, and sodium carbonate were purchased from sigma chemicals co (St Louis MO USA). All the chemicals used in were of analytical grade.

Plant material and preparation of leaf extract
The fresh leaf of *Terminalia arjuna* were collected during March 2019 from Rajendranagar, Hyderabad, India. The plant species were authenticated by Scientist, Agricultural College, Hyderabad, India. The fresh leaves of TA were washed twice with distilled water and shade

dried at room temperature for 40-45 days. Leaves were powdered using a mechanical blender and subjected to Soxhlet extraction. The extraction procedure was repeated successively with solvents of increasing polarity, petroleum ether, benzene, chloroform, acetone, hexane and aqueous. After extraction the extract was concentrated in a flash evaporation evaporator, solvent was recovered and the extract was dried in a desiccators and the extracts were stored in brown bottles at room temperature.

$$\text{Percent extractability} = \frac{\text{Total amount of extract obtained}}{\text{Total weight of powder taken for extract}} \times 100$$

Qualitative Phytochemical screening

Crude extracts of leaves were examined for the presence of various secondary metabolites such as phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterol, steroids and carbohydrates as described by (Raman 2006) [10].

Quantification of phytochemical constituents

The total phenolic content of leaves extracts was estimated according to the Folin Ciocalteu method (Sembiring *et al.,* 2018) [11] with minor modifications. Total phenolic content was calculated from calibration curve of Gallic acid (25-200ug/ml) and expressed in terms of Gallic acid equivalents (GAE) per gram of extracts. The standard solutions of Gallic acid concentrations 1.56-100ug/ml were prepared in water. 50ul of extract (1mg/ml) or standard solution were added to 50ul of distilled water. 50ul of 10% Folin Ciocalteu phenol reagent and 50ul 1M sodium carbonate solution were added to the mixture in a 96 well plate. Distilled water was used as blank. Reactions were incubated for 60 min at room temperature and protected from light. The absorbance was measured at 750nm with a microplate reader. Total phenolic contents were expressed as ug Gallic acid equivalents (GAE) per ml of plant extracts.

The total flavonoid content in different extract of leaves of Ficus religiosa were determined by using aluminium chloride method (8). The flavonoid content was calculated from standard curve of Quercetin (25-200ug/ml) and expressed as Quercetin equivalents (QE) per gram of extract. The total flavonoid content was determined by aluminium chloride calometric assay adopted from Sembiring *et al.,* 2018 [11]. Quercetin was used as a standard. Standard solutions of Quercetin of concentration 1.56-100 ug/ml were prepared in 80% ethanol. 50ul of extracts (1mg/ml) or standard solution was added to 10 ul of 10% aluminium chloride solution and followed by 150ul of 95% ethanol. 10ul of 1M sodium acetate was added to the mixture in a 96 well plate. 80% ethanol was used as reagent blank. All reagents were mixed and incubated for 40min at room temperature and protected from light. The absorbance was measured at 415nm with a microplate reader (Biotek, USA). Total flavonoid contents were expressed as ug Quercetin equivalents (QE) per ml of plant extracts.

Antioxidant scavenging activity

DPPH radical scavenging activity of different extracts was measured by Jose Prieto method 2002. DPPH scavenging ability assay was used to evaluate the antioxidant activity of each extracts and the test was conducted in a 96 well plate with slight modifications. 20ul stock solution of extracts in different concentrations (1.075 to 200ug/ml) and 180ul of DPPH solution (0.147mM) was added to each well. After 30min of incubation at room temperature in dark room, absorbance was read at 517nm using microplate reader. Methanol was used as blank. Ascorbic acid was used as positive standard. All tests were performed in triplicate. Concentration of samples resulting in 50% inhibition on DPPH (IC_{50} value) was calculated. The scavenging ability (%) was calculated as follows:

$$\%\ \text{inhibition} = \frac{\text{Absorbance of standard-Absorbance of extract}}{\text{Absorbance of standard}} \times 100$$

The effective concentration required for 50% reduction of the DPPH radical (IC_{50}) was calculated by probit analysis using IBM SPSS version 20.0.

Nitric oxide scavenging assay was carried out using sodium nitroprusside method (Sreejayan and Rao 1997) [13]. Gallic acid was used as positive standard. The assay is the Nitric oxide radical scavenging assay. The extracts were prepared from a 10 mg/ml ethanol crude extract and were serially diluted with distilled water to make concentrations from 100−1000µg/ml of the plants extracts and the standard Gallic acid and stored at 4 °C for later use. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.9% naphthyl ethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 ml of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 ml of the different concentrations of the various extracts (100-1000 ug/ml) and incubated at 25 ^{0}C for 180 min. The extract was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The colour tubes contained ethanol extracts at the same concentrations with no sodium nitroprusside. A volume of 150µl of the reaction mixture was transferred to a 96-well plate. The absorbance was measured at 546 nm using a microplate reader. Gallic acid was used as the positive control. The percentage inhibition of the extract and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the extracts and Gallic acid were calculated using the following formula:

$$\text{Percentage nitrite radical scavenging activity} = \frac{\text{A control} - \text{A Test}}{\text{A control}} \times 100$$

A control- Absorbance of control sample, A Test - Absorbance in the presence of samples of extract or standards

Statistical Analysis

Inhibition of concentration and total phenolic and antioxidant were determined by regression analysis method which was used to calculate IC_{50}.

Result

The extractive yield of different extracts of *Terminalia arjuna* leaf were ranged from 3-12gm/50g. The highest percentage yield in case of TALE was found in water (aqueous), whereas the lowest was in chloroform. The extracts of TAL revealed presence of phenols, saponin, tannins, phenols alkaloid, flavonoid whereas absence of alkaloid and glycosides in aqueous and chloroform extracts.

The total flavonoid content of *Terminalia arjuna* leaf was expressed as ug GAE/mg extract and was ranged from 44.686-263.41 ug QE/mg. Equation of calibration curve of Quercetin standard was y = 0.2292x+0.2441 R^2= 0.9989. The maximum amount of total flavonoid content (263.41 ug QE/mg) was obtained from the leaf extract prepared in aqueous and that of minimum (44.686 ug QE/mg) was from in Acetone. Among the six extracts, aqueous contained the highest amount (263.41 ug QE/mg) of the total flavonoid content compounds followed by benzene (142.949 ug QE/mg), hexane (94.834 ug QE/mg), petroleum ether (73.946 ug QE/mg), chloroform (67.809 ug QE/mg) and acetone (44.686 ug QE/mg).

The total phenolic content of extracts of *Terminalia arjuna* leaf expressed as ug GAE/mg extract and it was ranged from 37.696- 42.229 ug GAE/mg. The calibration curve from Gallic acid showed maximum absorbance at 765 nm wavelength (equation y= 0.0053x +0.0787, R2= 0.9931). The total phenolic content of six extracts of leaf of *Terminalia arjuna* determined by the Folin-Ciocalteau method was reported as Gallic acid equivalents (GAE). Among the six extracts, aqueous extracts contained the highest (42.229 ugGAE/mg) amount of the total phenolic content followed by benzene (41.685 ug GAE/mg), acetone (40.024 ug GAE/mg), petroleum ether (38.457 ug GAE/mg), hexane (38.239 ug GAE/mg), chloroform (37.696 ug GAE/mg) and Gallic acid (19.73 µg/mg).

Phenolic compounds including flavonoids are considered as the important antioxidative components of plant materials because of the positive correlation between the concentration of plant phenolic and its total antioxidant capacity (Barry Halliwell 1996) [6]. DPPH free radical scavenging method was used to determine the concentration of extract at which they scavenge the 50% of the DPPH solution termed as IC50 values. Ascorbic acid was used as a standard for this purpose. In terms of percentage, the inhibitory activity (at 30 min) of ascorbic acid, aqueous, acetone, benzene, chloroform, hexane and petroleum ether extracts of In terms of percentage, 45.99, 45.00, 45.49, 44.89, 45.84, 47.74 and 46.98% inhibition of activity (at 30 min) was found for ascorbic acid, aqueous, acetone, benzene, chloroform, hexane and petroleum ether extracts of *Terminalia arjuna* leaf at a concentration of 10.87, 11.11, 10.99, 11.13, 10.9, 10.47 and 10.64 ug/ml, respectively.

The trend of antioxidant activity was lesser for aqueous, acetone and chloroform extracts of *Terminalia arjuna* leaf, and was higher in petroleum ether followed by hexane and benzene. The maximum NO scavenging of aqueous, acetone, benzene, chloroform, hexane and petroleum ether of *Terminalia arjuna* leaf extract was 44.15, 45.77, 50.38, 45.8, 50.97 and 52.67%, respectively with IC_{50} values of 11.32, 10.92, 9.92, 10.91, 9.80 and 9.49 mg/ml, respectively. Nitric Oxide radical scavenging activity of all the *Terminalia arjuna* leaf extracts (aqueous, acetone, benzene, chloroform, hexane and petroleum ether) was lower compared to the standard Gallic acid.

Table 1: Extractive yield of different extracts of *Ficus Religiosa* and *Terminalia arjuna*

Solvent	Extractive yield (g/50g) TALE
Acetone	3.7
Aqueous	12
Benzene	9.0
Chloroform	3.0
Hexane	3.49
Petroleum ether	3.83

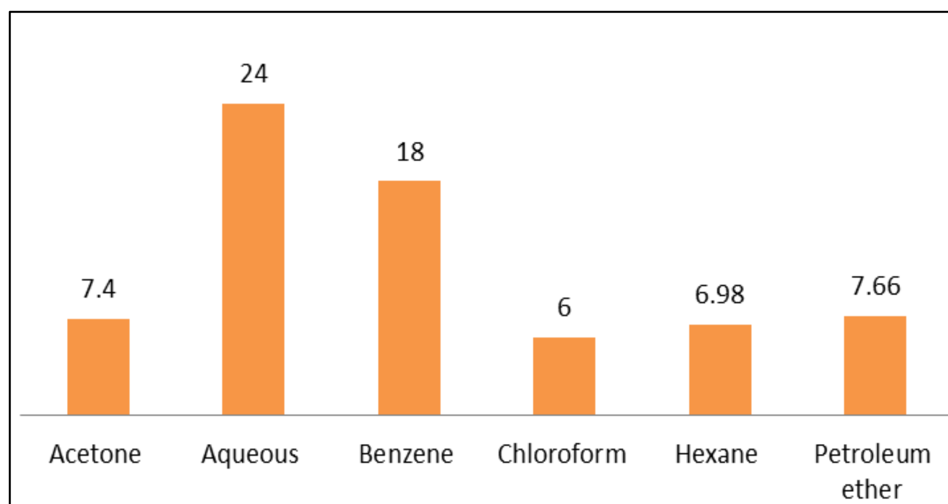

Fig 1: Extractability (%) of different extracts of *Terminalia arjuna* leaf

Table 2: Qualitative phytochemical screening of different extracts of *Terminalia arjuna* leaf

Phytochemical constituent	Aqueous	Acetone	Benzene	Chloroform	Hexane	Petroleum ether
Alkaloids	-	+	+	-	+	+
Glycosides	-	+	-	-	-	+
Flavonoids	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Triterpenoids	+	-	-_	+	-	+
Steroids	-	-	-	-	-	+
Saponins	-	+	-	+	-	-
Tannins	-	-	-	+	-	-
Carbohydrates	+	-	-	-	-	+
Proteins	+	+	+	+	+	+

able 3: Quantification of total flavonoid and total phenolic content in different extracts of *Terminalia arjuna* leaf:

Name of the extract	Total flavonoid content (µgQE/mg extract)	Total phenolic content (µgGAE/mg extract)
Aqueous	263.41	42.22
Acetone	44.68	40.02
Benzene	142.95	41.68
Chloroform	67.81	37.69
Hexane	94.83	38.24
Petroleum ether	73.94	38.45

Table 4: DPPH radical scavenging activity *of Ficus religiosa* and Terminalia arjuna leaf extract

Extract	IC_{50}(µg/ml) TALE
Ascorbic acid	10.87
Acetone	11.11
Aqueous	10.47
Benzene	11.13
Chloroform	10.99
Hexane	10.99
Petroleum ether	10.64

Table 5: Nitric oxide radical scavenging activity *of Terminalia arjuna* leaf extract:

Extract	IC_{50}(µg/ml) TALE
Gallic acid	14.26
Acetone	11.69
Aqueous	10.92
Benzene	9.85
Chloroform	9.92
Hexane	9.8
Petroleum ether	9.49

Discussion

Natural antioxidants such as flavonoids and phenolic compounds are believed to possess antioxidant properties due to their reducing and chelating capabilities (Barry Halliwell 1996) [6]. Flavonoids and phenols are secondary metabolites with free radical scavenging abilities that are widely distributed in fruits, leaves, bark and other parts of plants (Deyab *et al.,* 2016) [8]. The total phenolic content of the extracts was determined by Folin Ciocalteu reagent due to its high specificity for the polyphenolic compounds present in the plant extracts as it does not interact with the other phytochemicals due to due to complex formation between reducing species and phosphorus-molybdic tungstate (Jose Prieto 2001). It is evident from present study that leaf extracts of TA were good source of antioxidant.

In the present study, the highest percentage yield was found in water (aqueous), whereas the lowest was in chloroform extract of *Terminalia arjuna*. *Terminalia arjuna* leaf extracted with Phytochemical screening of TALE revealed the presence of alkaloids, tannins, saponins, glycosides, flavonoids, phenols, carbohydrates and reducing sugars in acetone, benzene, chloroform and hexane extracts, and there was absence of alkaloids, glycosides and saponins in aqueous and petroleum ether extracts. Our results are in agreement with the reports of who observed the presence of tannins, flavonoids, phenolic compounds, tannins, flavonoids, glycosides and phytosterols in benzene, hexane and petroleum ether extracts (Tahira and Sharma 2014, Singh *et al.*, 2018) [14, 17].

TA was used as antioxidant (Aditya Arya 2012) [18]. It is evident from the present study that TA was good source of antioxidants. The maximum total flavonoid content was found in aqueous extract followed by benzene, hexane, petroleum ether, chloroform and acetone extracts of TALE. Among six extracts of TALE, aqueous extracts contained highest amount of total phenolic content followed by benzene, acetone, petroleum ether, hexane and chloroform. The maximum total flavonoid content was found in aqueous extract followed by benzene, hexane, petroleum ether, chloroform and acetone extracts of TALE. Among six extracts of TALE, aqueous extracts contained highest amount of total phenolic content followed by benzene, acetone, petroleum ether, hexane and chloroform.

The present study investigation demonstrated that the antioxidant potential of leaf extracts of TA through inhibition of generation of free radicals in-vitro. The reactive oxygen species such as superoxide and hydroxyl radicals, hydrogen peroxide are often generated as product of biological reactions and damage the cells when present in excess. Both plant showed significant DPPH scavenging activity in dose dependent manner. The DPPH method is a stable free radical system and a sensitive way to determine the *in vitro* antioxidant activity of plant extracts. The antioxidant efficacy is associated with their scavenging ability of stable free radicals. The DPPH assay suggests that the extracts of TA are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for the radical reactions (Aditya Arya 2012, El-Ameen and Rafety 2013) [18,, 19]. The nitric oxide is another free radical, which acts as pleiotropic inhibitor of physiological processes and reacts with superoxide anion radicals to form a cytotoxic oxidant molecule, peroxynitrite. In the present investigation, TA effectively scavenged nitric oxide. The scavenging of NO by extracts was increased in a concentration-dependent manner and showed maximum activity in aqueous extracts. Phenolic compounds directly contribute to antioxidant action of the natural substances (Chatha *et al*., 2014) [15]. It is reported that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans and polyphenols are potential protecting agents against lethal effects of oxidative stress. In the present investigation the aqueous leaf extract of TA had the highest phenolic content concomitant with high free radicals scavenging action. Hence high antioxidant potential of TA extract might be due to presence of more phenolic and flavonoid content. The total phenolic and flavonoid in aqueous extract of *Terminalia arjuna* were more compared to that of other extracts. There is a direct correlation of phenolic and flavonoids with antioxidant activities in leaves which clearly indicate that phenolic compounds and flavonoids may be responsible for antioxidant activities of *Terminalia arjuna*. The present study validates the use of leaves of *Terminalia arjuna* for treatment of various ailments.

Conclusion

In conclusion, present study demonstrates the antioxidant capacity of all the four extract of *Terminalia arjuna* leaf. The finding of the present study specify the importance of medicinal plants as a readily available source of antioxidants

in order to prevent the occurrence of non-communicable diseases like cancer, diabetes, dementia and myocardial infarction for which the free radicals are considered one of the major contributing factors.

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References

1. Kamboj VP. Herbal medicine. Current science 2000;78:35-39.
2. Rice-Evans C, Miller N, Paganga. Antioxidant properties of phenolic compound. Trends Plant Science 1997;2:152-9.
3. Mandal S, Patra A, Samanta A, Roy S, Mandal A, Das T *et al*. Analysis of phytochemical profile of *Terminalia arjuna* bark extract with antioxidative and antimicrobial properties. Asian Pacific Journal of Tropical Biomedicine 2013;3:960-966.
4. Tripathi VK, Singh B. *Terminalia arjuna*- its present status. Oriental Journal of Chemistry 1996;12(1):1-16.
5. Udupa KN. Scope of use of Terminalia arjuna in ischemic heart disease. Annals of the National academy of Indian medicine 1986;1:54.
6. Berry Halliwell. Free radicals, proteins, and DNA: Oxidative damage versus redox regulation; Biochemical society Transactions 1996;24(4):1023-1027.
7. Chandan K, Raj K, Shamshan N. Phytochemical properties of total antioxidant status of acetone and methanol extract of *Terminalia arjuna* Roxb bark and its hypoglycemic effect on Type-II diabetic albino rats. Indian journal of pharmaceutical science 2013;2:199-201.
8. Deyab M, Taha C, Fatma W. Qualitative and quantitative analysis of phytochemical studies on brown seaweed, *Dictyola dichotoma*. International Journal of Engineering Development and Research 2016;4:674-678.
9. Khan WM, Khan SZ, Khan MS, Akhtar N. A preliminary phytochemical screening of medicinal plants: A case study of selected plant species at three phonological stages. Pakistan Journal of Weed Science and Research 2016;22:329-352.
10. Raman N. Phytochemical methods. New Indian publishing agencies, New Delhi 2006, 19.
11. Sembiring EN, Berna E, Sauriasari R. Phytochemical screening, total flavonoids and total phenols content and antioxidant activity of different parts of *Caesalpinia bonduc* (L) Roxb. Pharmacognosy journal 2018;10:123-127.
12. Jose Prieto. Dr. Prieto's DPPH microplate protocol 2012.
13. Sreejayan, Rao MN. Nitric oxide scavenging by Curcuminoids. Journal of pharmacy and Pharmacology 1997;49:105-107.
14. Tahira P, Sharma K. Phytochemical profiling of leaves and stems of *Terminalia arjuna* and *Tecomela undulata*. International journal of pharmacy and biosciences 2014;1:1-7.
15. Chatha SSA, Hussain AJ, Asad R, Majeed M, Aslam N. Bioactive components and antioxidant properties of *Terminalia arjuna* extracts. Journal of food processing technology 2014;5:1-5.
16. Christy S, Ravichnadran S, Prabhakaran M, Muthukumar R, Selvakumar S, Farzana J. *In-vivo* cytotoxic activity of Terminalia arjuna leaf extracts using brine shrimp and phytochemical screening. Asian Journal of Research in biological and pharmaceutical sciences 2015;3(3):95-102.
17. Singh V, Bharadwaj N, Wahj N, Tripathi VJ. Phytochemical analysis and *in-vitro* antimicrobial activities of *Terminalia arjuna* leaf, bark and fruit extracts in different solvents. Current trends in biotechnology and pharmacy 2018;12:286-294.
18. Aditya Arya, Shaikh N, Mohammed IN, Mohammed MA. Antioxidant and hypoglycemic activities of leaf extracts of 3 popular *Terminalia* sp. E-Journal of chemistry 2012;2:883-892.
19. El-Ameen SM, Rafety LA. Chemical investigation and antioxidant activity of phenolic acids from leaves of *Terminalia arjuna*. Global journal pharmacology 2013;7:448-456.
20. Ali A, Kaur G, Hamid H, Tarique AM, Ali N. Terminoside A, a new triterpene glycoside from the bark of *Terminalia arjuna* inhibits nitric oxide production in murine macrophages. Journal of Asian natural products 2003, 137-142.