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Evaluation of Phytochemicals, Antioxidant and Anti-Inflammatory Screening of *Terminalia arjuna*

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ABSTRACT

Objective: To investigate phytochemical screening, antioxidant, anti-inflammatory and cytotoxic activity of *Terminalia arjuna.* **Methods:** For phytochemical screening of flavonoids and phenols qualitative and quantitative analysis were carried out, while tannins, saponins and glycosides qualitative by some common methods with standards test were done. Detection of antioxidant activity was measured by 2,2-diphenyl-1-1picrylhydrazyal(DPPH) in colorimetry. The anti-inflammatory assay was done at Clinico lab, Mulund (E), Mumbai using whole human blood. **Results:** Phytochemical screening showed qualitatively and quantitatively presence of flavonoid and phenolic compound while it also exhibited presence of saponin, tannins and glycosides qualitatively. The promising antioxidant activity as absorption of DPPH radicles decreased in DPPH free radical scavenging assay. Anti-inflammatory activity revealed very less protection at 8% at 25mg/ml concentration. **Conclusion:** The *T. arjuna* bark extract revealed the presence of potent phytochemicals and anti-inflammatory bioactive constituents which are known to exhibit medicinal as well as physiological activity.

INTRODUCTION

Day by day there is exponential growth in the use of plants as potential herbal drugs to cure many infection and diseases. As per WHO, more than 80% of world's population depends on herbal medicine for primary healthcare (Pizzorno and Murray 2012). A large number of plants with therapeutic properties are quite astonishing. It is estimated that around 70,000 plant species, from lichens to trees, have been used at one time or another for medicinal purposes (Das *et al*., 2003). The continuous development of antibiotically resistant strains of microbial pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin- resistant enterococci (VRE), are growing problems and it is, therefore, extremely important to discover and develop new antimicrobial compounds (Tally *et al.,* 1999).

Terminalia arjuna is a deciduous tree found throughout India growing to a height of 60-90 feet. The thick, white-to-pinkish-gray bark has been used in India's native Ayurvedic medicine for over three centuries, primarily as a cardiac tonic. Terminalia's active constituents include tannins, cardenolide, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin, arjunglycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), phytosterols, calcium, magnesium, zinc, and copper (Bone, 1996 and Kapoor 1990)

Phytochemical studies reveals the presence of arjunosic acid, tomentosic acid, β-sitosterol, ellagic acid, leucodelphinidin Arjun glucoside I & II (Rastogi&Mehrotra 1993), arjunic acid , arjunetin (Row *et al.* 1970), arjunolitin (Tripathi et al. 1992), casuarianin (Kuo et al. 2005) in stem Bark while in roots bark arjunoside I&II, terminic acid (Anjaneyulu& Ram Prasad 1983), leucocyanidin, Gallic acid, ellagic acid (Anjaneyulu& Ram Prasad 1992) and fruits arjunone, β-sitosterol, Gallic acid, arachidic stearate (Rastogi & Mehrotra 1993), terminolitin (Singh *et al.* 1995).

Bioactive properties of *T.arjuna* is known from ages like antimicrobial (Shinde *et al.*, 2011), antidiabetic (Manna et al 2009a; 2009b) antiacne (Vijayalakshmi *et al.*, 2011) ,antiasthmatic (Prasad *et al.*, 2004;),cardioprotective (Radhakrishnan *et al.*, 1993) and many more properties this magical plant exhibit.

MATERIALS AND METHODS

Plant material (Bark) was collected from an ayurvedic shop from Mulund, Mumbai. Plant material of *Terminalia arjuna* was extracted using soxhlet extractor using ethanol as solvent for extraction. After extraction process was completed, the solvent was removed or recovered by means of rotary evaporator.

Phytochemical Analysis:

Flavonoid analysis was done by standard Alkaline reagent test using quercetin (5-100µgm/ml) was used as a standard. Absorbance was measured at 510 nm spectrophotometrically (Mandal *et al*. 2008) whereas phenolic compound analysis was carried out by ferric chloride test using gallic acid as standard and absorbance were measured at 765 nm spectrophotometrically (Satheesh Kumar *et al.* 2013*).*

Qualitative analysis of Tannins (Braemer's test) in which Extract was added (500 µl) in test tube and in control tube. The sample was sonicated using a sonicator for 5 minutes so that all samples should get mixed properly. Then 10% alcoholic $FeCl_3$ was added in test tube and color change was observed saponins (foam test) and glycosides (Horbone 1973).

Qualitative analysis of Saponins (Foam test): The stock solution was prepared in D/W and it was continuously mixed for 15 min. After mixing it properly, presence of froth was observed. (Horbone 1973) .

Qualitative analysis of Glycoside: The sample extract was prepared in D/W. The stock sample was mixed with chloroform and conc. H_2SO_4 after shaking for few seconds color change was observed. (Horbone 1973)

Antioxidant Assay:

Mandal method was used for antioxidant determination. Methanolic extract of plant material was used with varying concentration ranging from (1-100 ug/ml) for *T. arjuna*. Stock solution was added according to varying concentration in TEST & BLANK tube. After adding stock and diluents, DPPH reagent was added in dark in TEST and incubated for half an hour. Absorbance was measured at 517 nm spectrophotometrically. Ascorbic acid has been used as a standard. (Mandal *et al.* 2009**)**

The DPPH radical scavenging activity was calculated from the absorption value by the following equation

$$\% \text{ radical Scavenging} = \frac{\text{Absorbance of Control-(Absorbance of Test-Absorbance of Blank)}}{\text{Absorbance of Control}} X100$$

Anti-inflammatory assay:

Materials used for Anti-inflammatory assay was whole human blood obtained from Clinico Laboratory, Mulund (E). The blood sample was diluted by adding into approximately 50 ml of phosphate buffer saline. From the diluted sample, approximately 12-15ml of blood was drawn in centrifuge tube and it was subjected to centrifugation. After centrifugation, the supernatant was discarded and the pellet was again mixed with some amount of blood to get more concentrated RBC. Then absorbance was measured at 560 nm to check 8 x 10^9 cells/ml. This RBC sample was then used for the experimental purpose.

The method originally devised by N. Sampath Kumar (2011) was used with slight modification. Test sample for both the plants i.e. *T. arjuna* was prepared in (0.25%) hypotonic saline to produce a range of concentration (25-100µg/ml for *T. arjuna*). Aliquot was taken from the stock solution and various concentrations were prepared. After adding test sample, total volume was made up to 1ml by adding hypotonic saline. From this 25 µl of mixture was discarded. Rapidly 25µl of RBC suspension was added to each tube containing 8 X10^9 cells/ml and was shaken gently. Incubated at 45^0C for 30 minutes in water bath. After incubation, the reaction was terminated by a rapid high-speed centrifugation at 3500 rpm for 1 minute. After centrifugation, supernatant was transferred to another eppendorf tube and absorbance was measured at 560 nm. From the measured absorbance % protection for membrane, stabilization was determined.

RESULTS AND DISCUSSION

1. The qualitative test reveals the presence of following phytochemicals in *T. arjuna*

The current study also investigated the presence of phytochemicals like tannins, saponins and glycosides. Mandal *et al. (2009)* reported the presence of tannins, phenols, flavonoids, saponins and glycosides in *T. arjuna* and another investigator Ratha M *et al.* (2012) reported the presence of flavonoids, tannins and saponins in *H. indicus.*

Table- 1: Preliminary Phytochemical analysis of *T. arjuna*

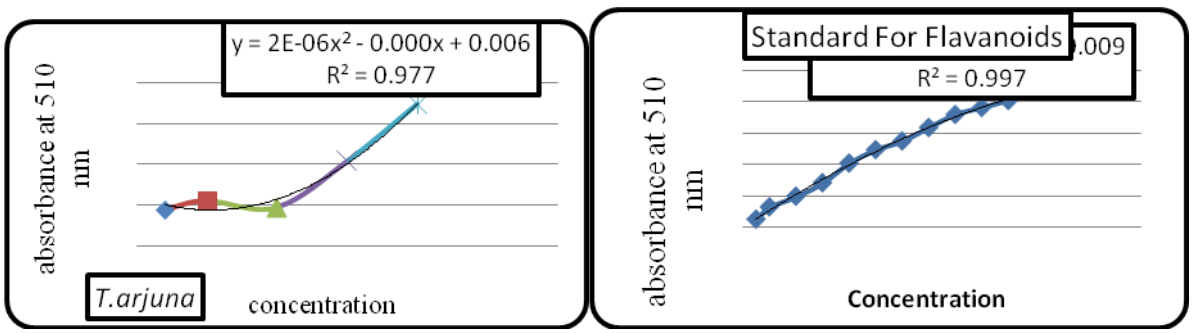
Sr. no.	Phytochemicals	Reaction
1	Flavonoids	+
2	Phenols	+
3	Tannins	+
4	Saponins	+
5	Glycosides	+

2. Estimation of Flavonoid

Table- 2 Alcoholic extract of *T. arjuna* for flavonoid content estimation

Concentration (µg/ml) standard Quercetin	Absorbance at 510nm	Concentration (µg/ml) of *T. arjuna*	Absorbance at 510nm
5	0.1209	10	0.0044
10	0.3171	25	0.0055
20	0.4864	50	0.0047
30	0.701	75	0.0104
40	1.015	100	0.017
50	1.2292		
60	1.3665		
70	1.5814		
80	1.7888		
90	1.9007		
100	2.0116		

Graph 1 a & b: Test and Standard graph for flavonoid using Quercetin (5- 100µg/ml)



The sample of plant used for estimating the presence of Flavanoids showed color change from colorless to yellow from lower concentration to higher concentration with a linear increase in graph. Change in color from lower concentration to higher concentration indicates the presence of Flavonoids in the sample.

Flavonoids are important group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in its structure (a range of C15aromatic compounds) and numerous reports support their use as antioxidants or free radical scavengers (Kar, 2007). The flavonoids content for *Terminalia arjuna* was reported by Sundar. S. Mety & Pratima Mathad (2011). It was observed from the results that the methanolic extract had higher content of flavonoids in stem bark of *T. arjuna* (0.90 ± 0.33 mg/100 gm). The present study for *Terminalia arjuna* was carried out and the absorbance was recorded to be 0.017 at 100 µg/ml for *T. arjuna* (Table 1 and Graph a &b).

3. Estimation of phenols

The sample used for estimating phenols showed color change from lowest concentration to highest concentration i.e. from 10-100 µg/ml for *T. arjuna* Also result was compared with standard (Gallic acid). Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to antioxidative action. The total phenolic content of *Terminalia arjuna* was reported by Sundar. S. Mety & Pratima Mathad (2011). It was found to be 1.9543±0.013 in ethanolic extract was reported by Satheesh Kumar et al. (2013). The present study for *Terminalia arjuna* was carried out and it was recorded to be 1.49 at 100µg/ml for *T. arjuna*.The result was compared with standard Gallic acid (5-100µg/ml) and was found to contain good amount of phenols in the extract.

Table- 3 Alcoholic extract of *T.arjuna* for phenol content estimation

Concentration (µg/ml) standard Gallic acid	Absorbance at 765nm	Concentration (µg/ml) of T.arjuna	Absorbance at 765nm
5	0.1209	10	0.0263
10	0.3171	25	0.0987
20	0.4864	50	0.2463
30	0.701	75	0.9705
40	1.015	100	1.4953
50	1.2292		
60	1.3665		
70	1.5814		
80	1.7888		
90	1.9007		
100	2.0116		

Antioxidant Assay

Antioxidant activity of *Terminalia arjuna* was determined by DPPH assay. There was color change observed in "test" from lower concentration to higher concentration for both the plants i.e. (1-100µg/ml) for *T. arjuna*. Standard used was ascorbic acid. R^2 value of the extract was compared with that of standard value and it was found to be similar to the test plant. The result obtained indicate that the plant possesses good amount of antioxidant activity (Table-4).

The methanol and ethanol extracts of *T. arjuna* showed maximum activity of 94.72 and 91.48%, respectively at 250 µg/ml, whereas ascorbic acid and BHT at the same concentration exhibited 96.66 and 92.59% inhibition respectively, reported by (Shahriar *et al.* 2012). The present study of ethanolic extract of *T. arjuna* showed maximum activity of 89.4% at 100 µg/ml whereas ascorbic acid exhibited 96.26% inhibition at 50 µg/ml.

Table- 4.Antioxidant assay of Alcoholic extract of *T. arjuna* by DPPH method

Concentration (µg/ml) Standard Ascorbic acid	% radical scavenging	Concentration (µg/ml) Test extract of *T. arjuna*	% radical scavenging
5	19.61	1	14.68
10	22.33	10	40.32
15	35.03	25	51.73
25	56.45	50	79.77
50	96.26	75	87.01
		100	89.4

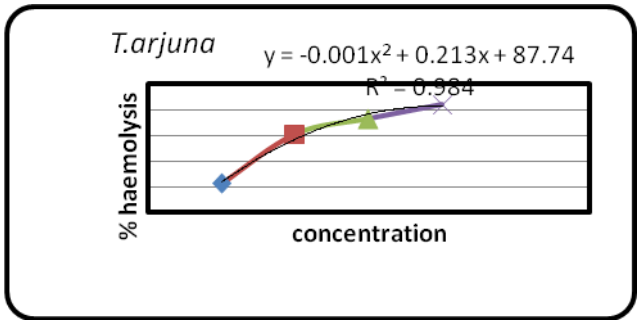
Anti-inflammatory assay

Anti-inflammatory assay was carried out by following the standard protocol. Plant material of *T.arjuna* revealed very less protection at lower concentration i.e. 8% protection at 25 µg/ml (Table 5). The result obtained indicates that the plant material used has very less anti-inflammatory activity at the concentration used.

Anti-inflammatory activity of *T. arjuna* was evaluated by Carrageenan-induced rat paw edema, (Biswas et al. 2013*).* In this study, the rats were divided into four groups which served as a control group- Carrageenan (Group I), (Group II) Carrageenan + Indomethacin, Group III & IV Carrageenan + META (Methanolic extract of *T. arjuna*).86.12 % inhibition was observed in Group II while 78.34 % & 80.70 % inhibition was observed in Group III & IV respectively.

Table 5: Result table and graph of % hemolysis at different concentration against concentration of *T.arjuna*

Concentration (ug/ml)	% hemolysis
25	92.27
50	96.09
75	97.26
100	98.37



CONCLUSION

The present study was carried out for "Evaluation of phytochemicals, antioxidant, anti-inflammatory and RBC hemolysis activity screening of *Terminalia arjuna.*

The present study reveals that the extract of *T. arjuna* was good source of phytochemicals which include flavonoids, phenols, tannins, saponins and glycosides. The study also indicated that the aqueous extract of plant material possibly prevents the effect of oxidative stress exhibiting % radical scavenging activity. This may be due to present of antioxidants such as flavonoids and other phenolic compounds and can be used as natural antioxidant and as a possible food supplement in pharmaceutical industries.Invitro study indicates the importance of plant extracts as a source of preventing the progress of various oxidative stresses.

Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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