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Comparative Evaluation of quality parameters in leaves, fruits and bark of *Terminalia* Sp

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Abstract
Terminalia sp belonging to Combretaceae family, is a tropical genus with 14 species in India. Out of that five species are used for praparation of medicine from ancient Ayurveda. Fruits of T. bellerica and T. chebula are the main ingredients in triphala churn while stem bark of T. arjuna, T. catappa and T. tomentosa have diuretic and cardiotonic properties. The present study was carried out for comparative evaluation of active phytoconstituents from *Terminalia sp*. Leaves, fruits and bark of five *Terminalia sp* were evaluated for total phenols, tannins and antioxidant potential. In case of leaves, fruitsand bark highest phenol content (10.31%, 22.38% & 12.35% resp), tannins (10.05%, 21.88% & 11.90 resp) and antioxidant potential (IC_{50} - 15.46, 35.73 & 37.46 resp) was observed in *T bellirica*. Qualitative HPLC was also carried out to know the presence of phenolic/tannin specific markers.

Keywords: *Terminalia sp*, phenols & tannins specific markers, antioxidant potential

Introduction

Terminalia is a tree species which is used as a medicine in India for more than 3000 years. These species are *Terminalia arjuna, Terminalia catappa, Terminalia tomentosa, Terminalia bellerica* and *Terminalia chebula.* [1] Tannins, flavonoids, and sterols have been identified in Terminalia species and other constituents include amino acids, fructose, resin and fixed oils.

Terminalia tomentosa also known as Indian Laurel which is deciduous tree upto 30 m height. Due to the characteristic bark pattern it termed as Crocodile Bark tree. Evaluation of the bark with ehanolic extract found four compounds which includes 4-methyl – 4 –hydroxymethylene - 6β - (10 -methyl octanyl), cyclohexane (Arjunahomosesquiterpenol), di-n-octyl phthalate, di isobutyl phthalate and dibutyl phthalate. [2] *Terminalia chebula* commonly known as black myrobalan which is native to South Asia from India and Nepal east to southwest China. It is also called as King of Medicine in Tibet. Based on the region where the fruit is harvested, as well as the colour and shape of the fruit these are of seven types viz. vijaya, rohini, putana, amrita, abhaya, jivanti, and chetaki. Fruits are used in tanning leather and dyeing cloth. It is the main ingredient in the Ayurvedic formulation *Triphala*, which is used for kidney and liver dysfunctions and dried fruits in Ayurveda as a purported diuretic, laxative, antitussive, cardiotonic and homeostatic.

Terminalia bellirica is a large deciduous tree to 50 m tall; branchless up to 20 m. Bark of the tree is bluish or ashy-grey covered with numerous fine longitudinal cracks and the inner bark yellowish. T. bellirica tree sheds leaves in November flowers in October-November and fruiting in November- December. Fruit extracts have anti-bacterial, germicidal activity and fruit rind is anthelmintic, pungent, astringent, laxative and antipyretic. [3] It is used in a diverse range of conditions including diarrhoea, dysentery, cough, tuberculosis, flatulence, liver disease, leprosy, eye diseases, anti-HIV-1, dyspepsia, inflammation of the small intestine, cleanse the blood and promote hair growth in the Ayurvedic drug. Tropical almond (*Terminalia catappa*) is a large, spreading tree now distributed throughout Indian Ocean, Tropical Asia and into the Pacific Ocean [4]. Fruits are produced from about 3 years of age which are nutritious, tasty may be eaten immediately after extraction. The leaf and bark extract use as a medicine for diaphoretic, anti-indigestion and anti-dysentery. In Philippines to cure headache and colic young leaves are used.

The thick white-to-pinkish-gray bark of *Terminalia arjuna* has been used for preparation of Ayurvedic medicine for over three centuries, specially for cardiac diseases.

It is a deciduous tree found throughout India growing to a height of 60-90 feet. Clinical evaluation proved that this botanical medicine benefit in the treatment of heart failure, possibly hypercholesterolemia, coronary artery disease and also act as antiviral and antimutagenic Terminalia's active constituents include tannins, cardenolide, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin, arjun glycosides), flavonoids (arjunone, arjunolone,luteolin), gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), phytosterols, calcium, magnesium, zinc, and copper. [5, 6] The bark has been described as an astringent, demulcent, expectorant, cardiotonic, styptic, antidysenteric, urinary astringent, and has shown to be useful in fracture, ulcers, leukorrhea, diabetes, anemia, cardiopathy, and cirrhosis.

Materials & Methods

The various plant materials such as the leaves, fruits and bark were harvested from different five species of *Terminalia* - *Terminalia arjuna*, *Terminalia catappa*, *Terminalia tomentosa*, *Terminalia bellerica* and *Terminalia chebula*, available at Nagarjun Medicinal Plants Garden, Dr PDKV, AKola (MS). Then samples were shed dried and powdered for further analysis of phenols, antioxidant potential and tannin content.

Estimation of Total Phenols & Tannins [7]

200 mg of powdered drug was extracted with 70% acetone for one hr on rotary shaker at 120 rpm, centrifuged at 3500g for 10 min, the supernatant was used for further estimation. Standard curve was prepared using 2,4,6,8 and 10 µg of tannic acid. 5 µl of sample (extract) was diluted to 0.5 ml with distilled water to which 0.25 ml of FC reagent was added followed by addition of 1.25 ml of sodium carbonate (20%). After vortexing, the absorbance was measured at 725 nm after 40 min incubation. Total phenol was calculated as tannic acid equivalent from standard curve and expressed on a dry matter basis (X%). To 100 mg PVPP, 1 ml distilled water was added followed by 1 ml extract, vortexed, kept on ice for 10 min, centrifuged for 3000g for 10 min, collect the supernatant. 10 µl was then diluted to 0.5 ml with distilled water to which 0.25 ml of FC (1N) reagent was added followed by addition of 1.25 ml of sodium carbonate. After vortexing, the absorbance was measured at 725 nm after 40 min incubation. Total non-tannin phenols were calculated expressed on a dry matter basis (Y%).

% Tannins (as TA equivalent) = Total Phenols (X%) – Non Tannin Phenols (Y%)

Estimation of Antioxidant Potential [8, 9]

Stock solution of samples were prepared by extracting 100 mg of dried powder in 100 ml of ethanol to give conc of 1mg/ml. Dissolved 0.5 g gallic acid in 10 ml ethanol and then diluted to 100 ml with water (5 g/liter final). 2, 4, 6, 8, and 10 ml aliquot was taken and diluted to 100 ml with water to create standards with 100,200, 300,400 and 500 mg/liter concentrations, respectively. Preparation of DPPH solution:- 4.3mg of DPPH was dissolved in 3.3 ml ethanol. 150µl DPPH solution was added to 3ml ethanol and absorbance was taken immediately at 516 nm for control reading. Different volume level of test sample (10, 20, 30, 40, 50µl) were screened and made 100µl of each dose level by dilution with ethanol. Diluted with ethanol upto 3 ml. 150µl of DPPH was added to each tube. Absorbance was taken at 516 nm after 15 min using ethanol as a blank. The median inhibitory concentration IC_{50} was calculated based on 50% inhibition of free radical formation.

HPLC Method

Qualitative HPLC was performed for presence of phenol specific markers using column: RP-18 (4.6 x 250 mm, 5 µm), Solvent A- Acetonitrile and Solvent B-TFA (0.05%) water - in gradient mode elution, flow rate of 1 ml / min, and PDA as detector. The HPLC analysis was performed at Project Directorate, AICRP on Medicinal, Aromatic Plants & Betel vine, (DMAPR-ICAR) Boriavi-Anand

Result & Discussion

Phytochemical analysis of plant revealed the presence of constituents which are known to have medicinal as well as physiological activities. [10] Many reports have already discussed the potential antioxidant activity in medicinal plants that is contributed by phenolic compounds [11, 12]. Phenolics are most ubiquitous groups of secondary metabolites found throughout the plant kingdom. An antioxidant is a molecule which can terminate the chain reaction by removing the free radical intermediates. There is a worldwide increase in concern towards the use of natural phytochemical based antioxidants. There are numerous reports on the web regarding potential benefit of antioxidant foods in preventing diseases.

In order to compare the total phenolics, total tannins and antioxidant activity, the authenticated trees of *Terminalia sp* available at Nagarjun Medicinal Plants Garden, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola (MS) were used. Routine biochemical protocols were used for total phenols and tannins.

As shown in Table-1, in case of leaves, highest phenol content and antioxidant activity was observed in *T. bellirica* (10.31% & IC_{50} 30.64 µg/ml) while lowest was *T. tomentosa* (5.16% & IC_{50} 37.17 µg/ml). In fruits, highest phenol content was observed in *T. bellirica* (22.38%) followed by *T. chebula* (19.78%) while antioxidant activity was highest in *T. chebula* (IC_{50} 13.06 µg/ml) followed by *T. bellirica* (IC_{50} 13.78 µg/ml). The lowest phenol content and antioxidant activity was observed in *T. catappa* (2.83% & IC_{50} 38.40 µg/ml). In the bark, highest phenol content and antioxidant activity was observed in *T arjuna* (12.35% & IC_{50} 26.44 µg/ml) followed by *T chebula* (11.76% & IC_{50} 29.27 µg/ml). The lowest content was observed in *T catappa* (7.97% & IC_{50} 37.46 µg/ml).

Qualitative HPLC was performed using C-18 column to know the presence of various phenolic/tannin specific markers such as gallic acid, corilagin, chebulagic acid, ellagic acid and chebulanic acid in various tissues of each *Terminalia sp* and the data is summarized in Table 2. As per Table-2 & Fig-1, gallic acid is present in all samples except *T chebulla* leaf. Ellagic acid is present in all samples except *T arjuna* fruit and *T catappa* bark. Corilagin is absent in all samples. Chebulagic acid is present in only *T chebulla* fruit and *T bellerica* leaf. Chebulanic acid is present in *T chebulla* fruit, *T bellerica* fruit and *T bellerica* leaf.

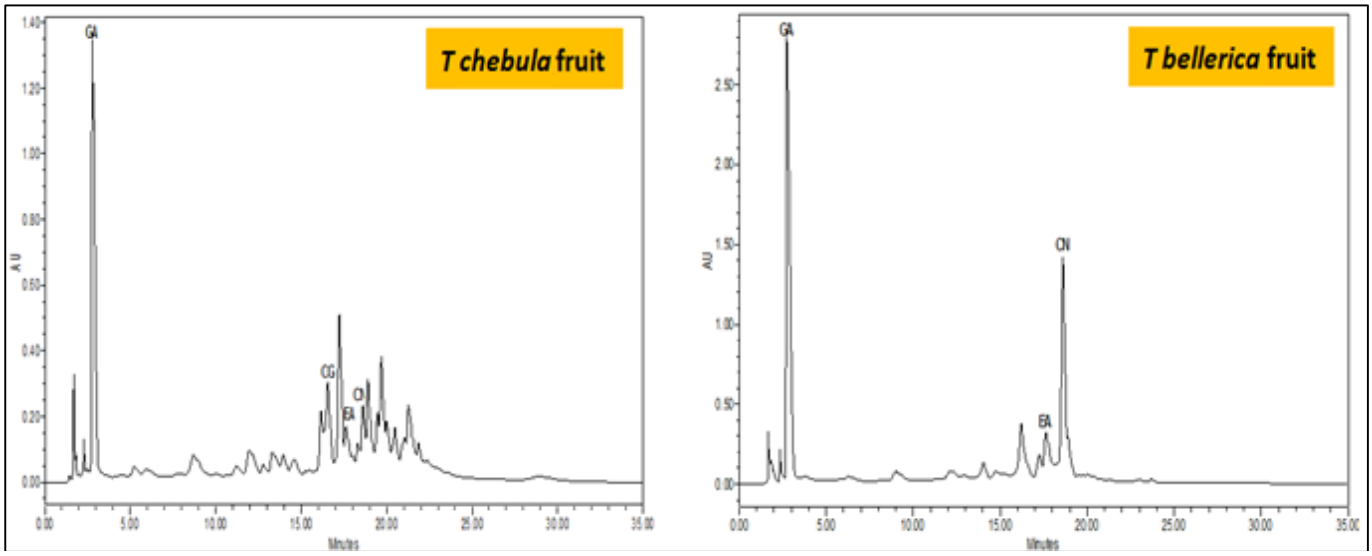
Table 1: Total phenols, tannins and antioxidant potential of *Terminalia sp*

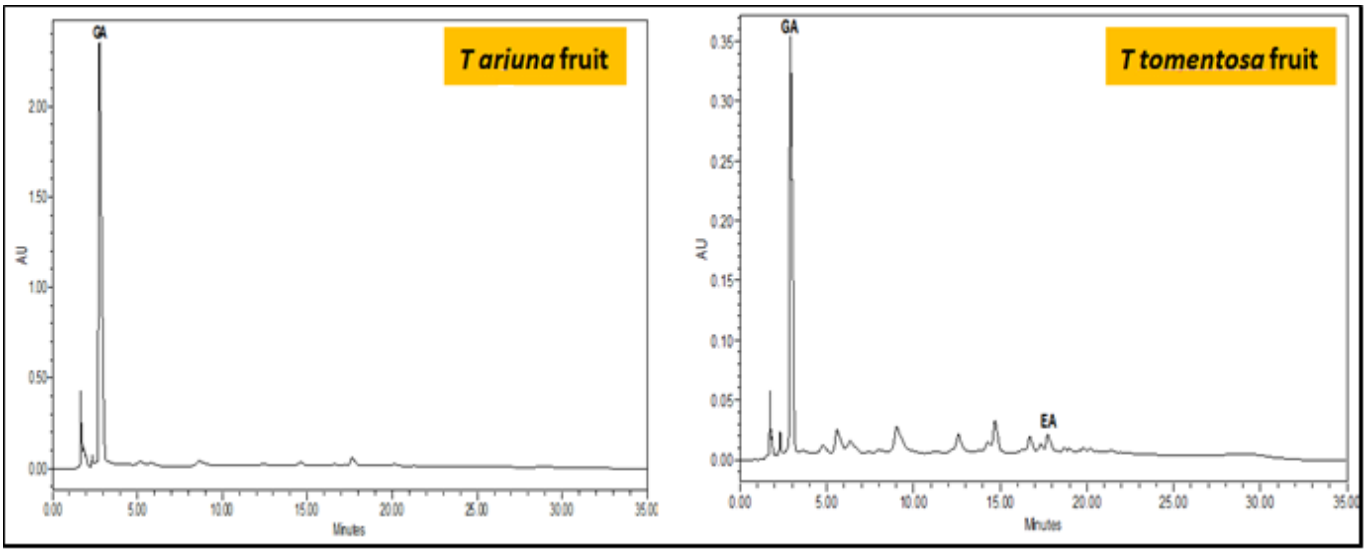
SN	Sample	*Terminalia sp*	% Total Phenols	% Total Tannins	Antioxidant Assay DPPH IC_{50}
1.	Leaves	*T arjuna*	7.73 ± 0.29	6.58 ± 0.23	35.78 ± 0.94
		T bellirica	10.31 ± 0.42	10.05 ± 0.65	30.64 ± 1.06
		T chebula	9.96 ± 0.64	9.81 ± 0.22	33.92 ± 0.41
		T tomentosa	5.16 ± 0.13	3.94 ± 0.41	37.17 ± 1.27
		T catappa	6.75 ± 0.19	4.65 ± 0.53	37 ± 0.46
2.	Fruits	*T arjuna*	5.68 ± 0.27	5.00 ± 0.17	36.81 ± 1.38
		T bellirica	22.38 ± 0.89	21.88 ± 0.81	13.78 ± 0.72
		T chebula	19.78 ± 0.63	18.34 ± 0.72	13.06 ± 0.96
		T tomentosa	5.67 ± 0.54	4.47 ± 0.37	36.05 ± 0.33
		T catappa	2.83 ± 0.13	2.78 ± 0.18	38.40 ±1.03
3.	Bark	*T arjuna*	12.35 ± 0.33	11.90 ± 0.37	26.44 ± 1.09
		T bellirica	6.97 ± 0.92	5.77 ± 1.07	34.64 ± 0.69
		T chebula	11.76 ± 0.95	11.25 ± 0.87	29.27 ± 0.63
		T tomentosa	8.63 ± 0.24	8.35 ± 0.21	33.12 ± 0.27
		T catappa	7.97 ± 0.34	7.62 ± 0.32	37.46 ± 0.42

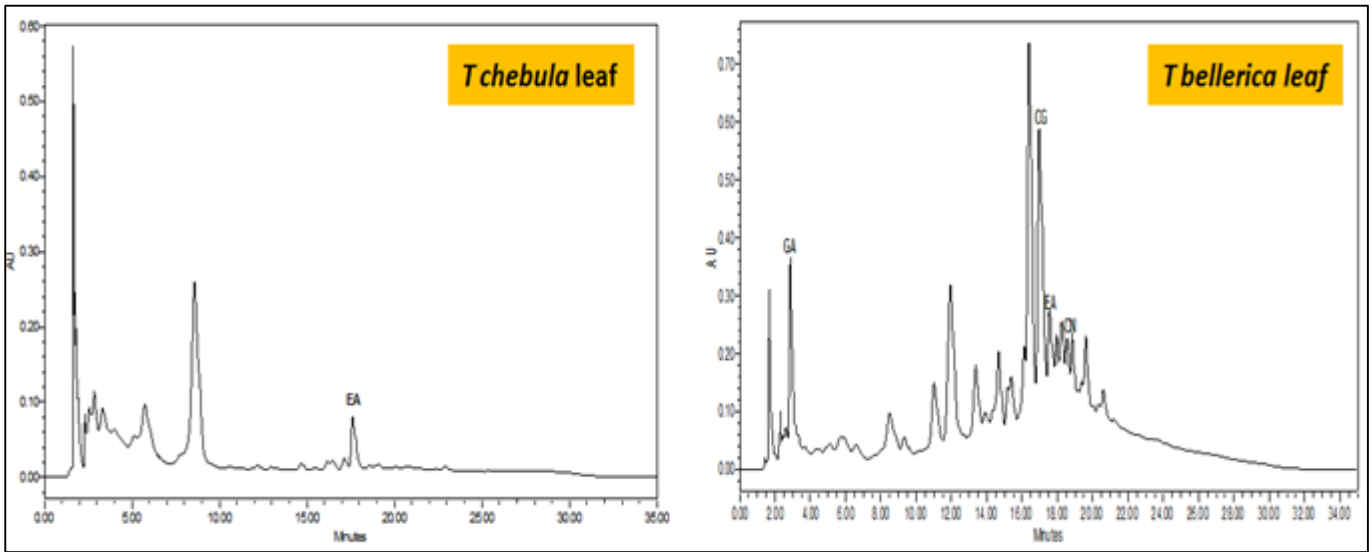
Values are mean of triplicate ± SD, IC 50 of Gallic acid= 12.43 µg/ml

Table 2: Qualitative HPLC Analysis of *Terminalia sp* for phenols/tannin related markers

Sample No	Terminalia sp	Gallic acid (GA)	Corilagin (CL)	Chebulagic acid (CG)	Ellagic acid (EA)	Chebulanic acid (CN)
1	*T chebula* fruit	√	X	√	√	√
2	*T bellerica* fruit	√	X	X	√	√
3	*T arjuna* fruit	√	X	X	X	X
4	*T catappa* fruit	-----NA--------				
5	*T tomentosa* fruit	√	X	X	√	X
6	*T chebula* leaf	X	X	X	√	X
7	*T bellerica leaf*	√	X	√	√	√
8	*T arjuna* leaf	√	X	X	√	X
9	*T catappa* leaf	√	X	X	√	X
10	*T tomentosa* leaf	√	X	X	√	X
11	*T chebula* bark	√	X	X	√	X
12	*T bellerica bark*	√	X	X	√	X
13	*T arjuna* bark	√	X	X	√	X
14	*T catappa* bark	√	X	X	X	X
15	*T tomentosa* bark	√	X	X	√	X




T ariuna fruit
GA
AU
Minutes
T tomentosa fruit
GA
EA
AU
Minutes


T chebula leaf
EA
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T bellerica leaf
GA
CG
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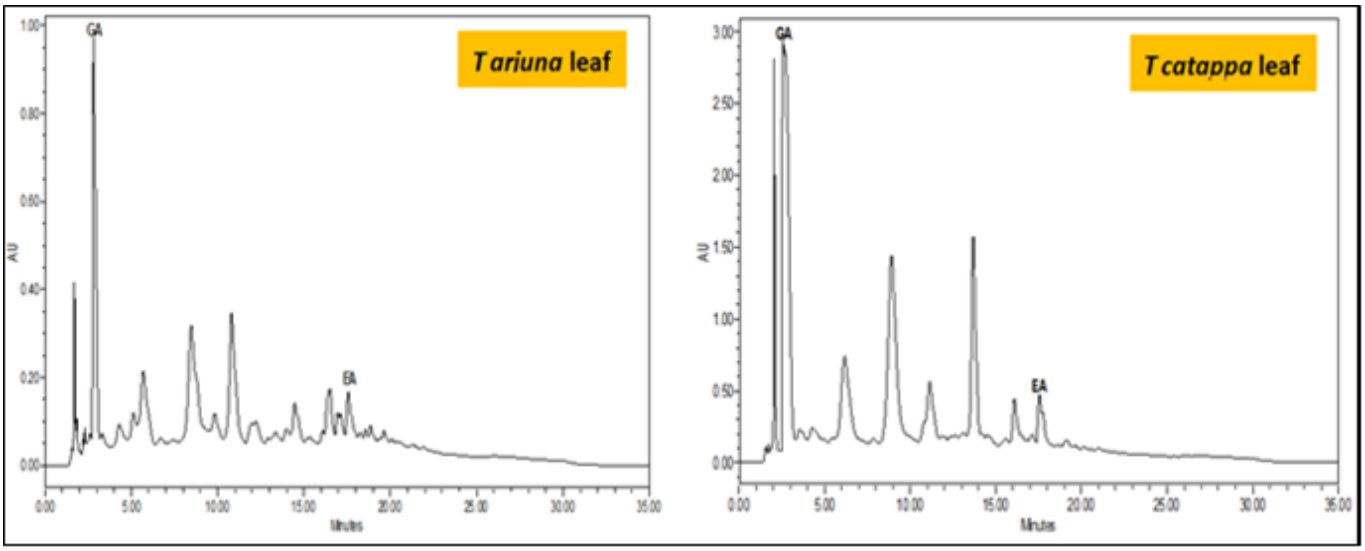
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T catappa leaf
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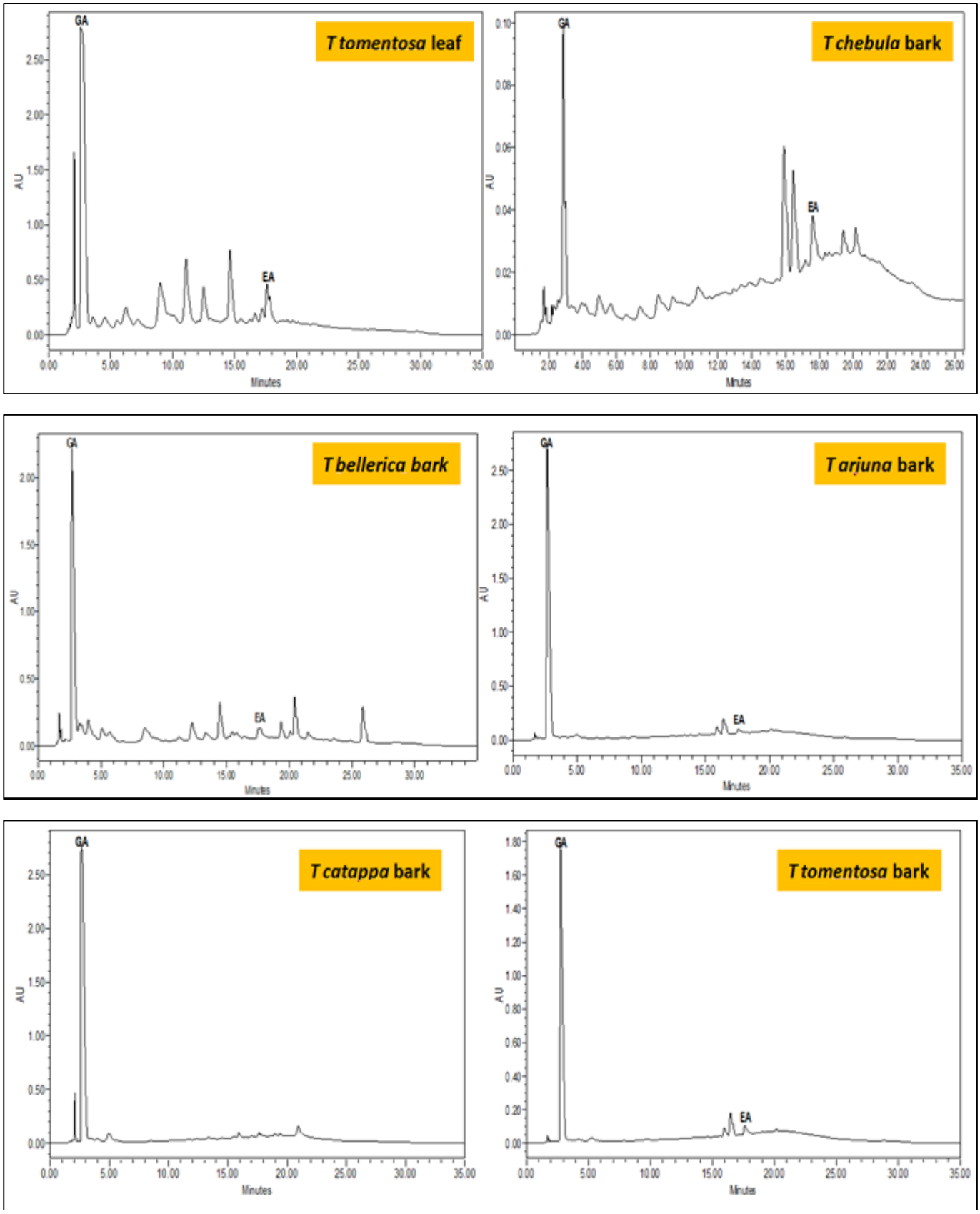

Fig 1: HPLC Analysis of *Terminalia sp*.

Conclusion

Terminalia sp is rich in phenols and hence antioxidant potential. The results concluded high phenol, tannin and antioxidant activity in *Terminalia sp.* Qualitative HPLC analysis also indicates the presence of phenol specific marker in *Terminalia sp*. The phenol and antioxidant activity was observed highest in leaves and fruits of *T bellerica* while in case of bark, the activity was more in *T arjuna*.

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