

Phytochemical Analysis of Selected Medicinal Plants from Eastern Ghats of Andhra Pradesh

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The objective of the present investigation is to appraise the qualitative and quantitative phytochemical analysis, DPPH and Hydroxyl radical scavenging activity along with a total antioxidant capacity of water extract obtained from six medicinal plants, i.e., *Acalypha alnifolia*, *Caesalpinia bonduc*, *Carissa spinarum*, *Commiphora caudata*, *Moringa concanensis* and *Terminalia tomentosa*. The phytochemical analysis of the six medicinal plants water extracts revealed total phenolic content (TPC) in the range of 190 to 25 GAE mg/g dwt. The highest TPC is present in *A. alnifolia* and the lowest is noticed in *T. tomentosa*. The total flavonoid content is present in between 80 to 12 QE mg/g dry weight and a higher amount of flavonoid content was observed in *A. alnifolia* and lowest present in *C. bonduc*. Antioxidant activity results of the six medicinal plants showed that the highest total antioxidant capacity was observed in *Carissa spinarum* and lowest in *T. tomentosa*. DPPH method was used to know the antioxidant capacity of aqueous extract of the six medicinal plants. Among the tested plants *A. alnifolia*, *Carissa spinarum* and *Moringa concanensis* strongly reduced DPPH purple color by expressing ~80% as maximum inhibition. DPPH was strongly inhibited by *T. tomentosa* (IC₅₀ value 25 µg/ml). Hydroxyl radical was strongly (~96%) inhibited by *T. tomentosa* and lowest IC₅₀ value was expressed by *A. alnifolia* (36.4 µg/ml). The study results indicate that all six medicinal plants are rich sources of natural antioxidant components.

Keywords: Aqueous extract, antioxidant studies, Eastern Ghats.

Free radicals are fragments of molecules with a very short half-life, generated through internal/ external sources, are highly reactive and damage macromolecules like proteins, DNA and lipids of cell membranes of living organisms. The most common reactive oxygen species are hydroxyl (OH), hydrogen peroxide (H₂O₂), super oxide anion (O₂⁻), and peroxy radicals (ROO) and nitrogen derived free radicals are nitric oxide

(NO), peroxy nitrite anion (ONOO), Nitrogen dioxide (NO₂) and Di-nitrogen trioxide (N₂O₃). The chemical constituents that retard or suppress oxidation or prolong the life of the oxidizable molecules/ inhibit the oxidation process are called antioxidants. Antioxidants from natural/ plant sources enhance the endogenous enzymes' anti-oxidative capability and reduce the risk of many free radical mediated diseases. Traditional

medicinal systems use medicinal plants containing various chemical components such as alkaloids, polyphenols, glycosides and terpenoids, which showed pharmacological properties such as antioxidant and antimicrobial activities^{1,2}. Of the estimated 2.5 to 5 lakhs plant species; very few have been screened for its biological or pharmacological activities³. In this connection, we selected the following water extract of six medicinal plants to screen phytochemical analysis and antioxidant potential.

Acalypha alnifolia Klein ex Willd., is a rare medicinal plant found in the forests of South India⁴. *Acalypha* belongs to the family Euphorbiaceae and is the fourth largest genus with about 450 species^{5,6}. In traditional medicine, the plant has been used to treat dysentery, diabetes and as mosquito repellent^{4,7,8}.

Caesalpinia bonduc Roxb. (family: Caesalpiniaceae) is locally known as Lata Karanja, is a prickly woody liana distributed throughout the hotter parts of India and Sri Lanka⁹. It is a valuable medicinal plant and its different parts, such as bark, leaves, roots and seeds are utilized in traditional system of medicine. The roots are effective as an antiperiodic and antispasmodic properties¹⁰, the bark is a good remedy as anthelmintic and febrifuge and the leaves were reported as an emmenagogue¹¹. The seeds are reported to have various pharmacological actions like antipyretic, antiperiodic, asthmatic and febrifuge^{12,13}.

Carissa spinarum L. is a spinous evergreen shrub that belongs to the family Apocynaceae, distributed throughout dry localities in India. The plant has been used in the Ayurvedic medical system to treat liver problems, epileptic disease, microbial infections, cytotoxic, viral diseases, inflammation, arthritis and cancer¹⁴. The ripened fruits are edible and reported to have cardiac protective properties.

Commiphora caudata (Arn) Engl. belongs to the family Burseraceae, is a thorny shrub to medium sized tree distributed in dry deciduous forests of Peninsular India of Andhra Pradesh, Karnataka and Tamil Nadu^{15,16}. It is commonly known as a hill mango because of the mango flavour of its stem bark. Traditionally, the bark has been used to treat diabetes, arthritis and obesity^{17,18}. Fruits have been used to prepare pickles and gum is used as incense.

Moringa concanensis Nimmo, is a rare medicinal plant of the family Moringaceae. It is found in dry localities in Konkan, Andhra Pradesh. In Telugu is called Konda munaga/ Karumunaga, because of its similar morphological characters of drumstick plant *M. oleifera*¹⁹. Different parts of this plant have been reported to treat different human ailments such as leaves for gynec problems, hyper-tension, constipation, jaundice, skin cancers, diabetes and splenomegaly, stem bark for abortion and fruits for rheumatism, nervous disorders, curing liver and spleen diseases, gum for dental problems and flowers for aphrodisiac, leucorrhea and abortion²⁰.

Terminalia tomentosa is a deciduous tree that belongs to the family Combretaceae. Generally, *T. tomentosa* is called a “crocodile bark tree” because the bark of this has characteristics feature like the skin of crocodile. It is found in Southern parts of the Indian subcontinent and other Southeast Asian countries²¹. In Ayurveda, the bark treats rheumatism, fever, urinary diseases and diabetes, vertigo, piles, constipation and chronic dysentery²².

MATERIALS AND METHODS

Plant materials

Selected medicinal plant parts *i.e.* *A. alnifolia* (aerial parts), *C. bonduc* (seeds), *C. spinarum* (fruits), *C. caudata* (stem bark), *M. concanensis* (leaves) and *T. tomentosa* (stem bark) were collected from Nallamala forests of Kurnool District, Andhra Pradesh, India. (Fig.1). The voucher specimens were deposited in Rayalaseema University Herbarium, Kurnool, Andhra Pradesh, India and were identified with the help of regional²³ and local floras²⁴.

Preparation of water extracts

The plant parts were cleaned with tap water, sliced and shade dried. The mixer grinder was used to grind the dried material to fine powder. The powdered material was mixed with double distilled water and boiled for 30 min and filtered. The filtrate was concentrated on water bath. The phytochemical and antioxidant activities of crude water extract (WE) were analyzed.

Preliminary Phytochemical Screening

Qualitative phytochemical screening

of the selected six medicinal plant extracts was analyzed using standard methods²⁵.

Total phenolic content

Folin-Ciocalteu (FCA) reagent method was used to estimate the total phenolic content of the water extracts in the six medicinal plants²⁶. The detailed procedure was followed as described²⁷.

Total flavonoid content

Aluminum chloride method was used to estimate the total flavonoid content (TFC) of the water extracts of the six medicinal plants²⁸. The detailed procedure was followed as described²⁷.

Ammonium molybdate dependent antioxidant capacity

Ammonium molybdate dependent total antioxidant capacity of the selected medicinal plant extracts was estimated by²⁹. The detailed procedure was followed as described²⁷.

DPPH and Hydroxyl radical scavenging activity

DPPH and Hydroxyl radical activity of water extracts were measured as mentioned in detail elsewhere^{30,31}. The detailed procedure was followed as described²⁷.

Table 1. Qualitative phytochemical analysis of water extracts of selected medicinal plants

Type of the Component	Selected Medicinal Plants					
	AA	CB	CS	CC	MC	TT
Alkaloids	+++	+	++	+	+	+
Anthraquinones		-	-			
Coumarins	++	+	+	+	++	+
Catecholic compounds	T	+	+	-		++
Glycosides	+	T	+	-	+	+++
Flavonoids	+	++	++	+	+++	++
Saponins	++	+	+	-	-	++
Steroids	-	-	-	T	-	-
Tannins		+	+	+	-	++
Terpenoids	+	+	-	++	+	+
Phenolic compounds	++	++	++	++	+	+++



Acalypha almifolia (Flowers)



Caesalpinia bonduc (Fruits)



Carissa spinarum (Fruits)



Commiphora caudata



Moringa concanensis (Flowers)



Terminalia tomentosa (Stem bark)

Fig. 1. Photographs of the selected medicinal plants

RESULTS

Qualitative Phytochemical Screening

The preliminary phytochemical studies on six selected medicinal plants revealed the presence of ten different secondary metabolites. Among the tested components, alkaloids, flavonoids, terpenoids, phenolic, tannins and glycosides showed strong reactions. At the same time, steroids showed very feeble reactions. Anthraquinones showed negative results in the tested plants.

Quantitative estimation of total phenolic/ flavonoid content

The results on total phenolic content in the selected medicinal plants are between 190 to 25 GAE mg/g dwt. The highest TPC is present in

TT and the lowest is noticed in CB (Figure 1). The total flavonoid content is between 80 to 12 QE mg/g dwt. The flavonoid content was higher in TT and lowest present in CB (Figure 2). Gallic acid and quercetin were used as standards.

Total antioxidant capacity (TAC)

The results on TAC of the selected medicinal plant extracts indicate that they expressed 240 to 40 ASE mg/g dwt. The highest TAC was observed in TT and the lowest in CB (Figure 3). Ascorbic acid was used as a standard component.

DPPH scavenging activity

The results revealed that all the plants expressed concentration dependent DPPH quenching activity. The plants, AA, CS and MC reduced DPPH purple color strongly by expressing

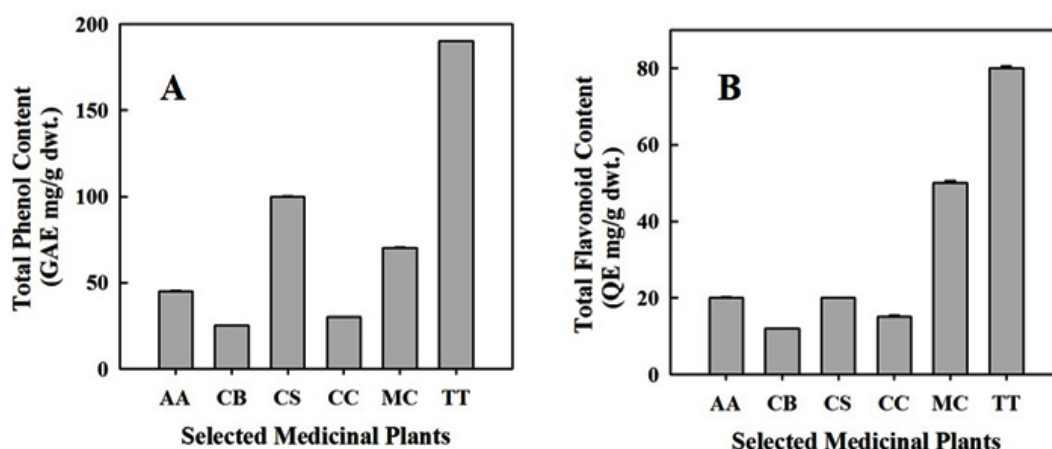


Fig. 2. Total Phenolic (A) and Flavonoid content (B) of water extract of selected medicinal plants

~80% as maximum inhibition. The extracts exhibited IC_{50} values to inhibit 50% of the DPPH radical between 300 to 25 μ g/ml (Figure 4).

Hydroxyl radical scavenging activity

All the plants tested exhibited concentration dependent hydroxyl radical scavenging activity (Fig. 5). Among the test plants *A. alinifolia* showed lowest IC_{50} value (36.4 μ g/ml) and *T. tomentosa* showed ~96% as maximum inhibition (Figure 5).

DISCUSSION

Recently researchers have been interested in exploring natural antioxidant principles that are therapeutically potent and with minimum low or no side effects to treat various human ailments

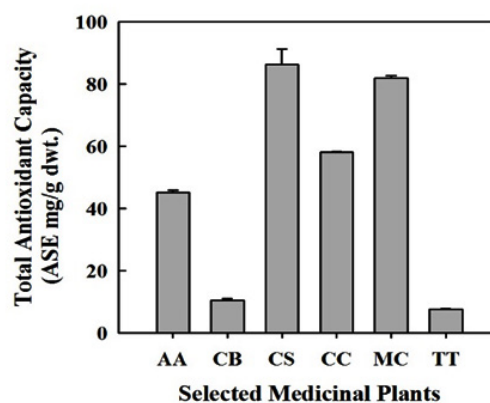


Fig. 3. Total antioxidant capacity (TAC) of water extract of selected medicinal plants

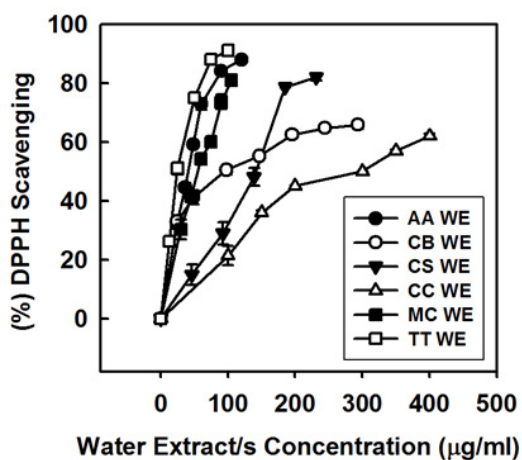


Fig. 4. DPPH quenching activity of water extract of selected medicinal plants

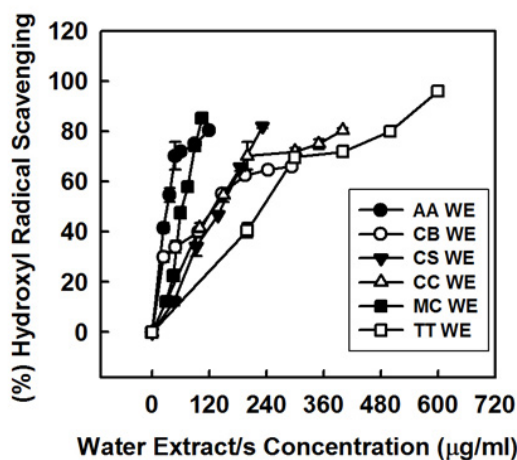


Fig. 5. Hydroxyl radical scavenging activity of water extract of selected medicinal plants

and for the food industry. Plants synthesize a wide variety of chemical components such as alkaloids (nitrogenous compounds), terpenes (lipid derivatives) and phenolics (carbohydrate derivatives) with potential pharmacological properties³². Thousands of biologically active phytoconstituents are isolated from higher plants. Of which phenolic compounds are phenolic acids, vitamin E, coumarins, flavonoids, isocoumarins, biflavonols, stilbene, phenols, quinones, betacitric, and chromones etc. reported exhibiting intense antioxidant activity. Scientifically phenolic and flavonoids are reported to have various pharmacological activities such as antioxidant, antiulcer, antispasmodic, cytotoxic anti-inflammatory, antitumor, and antidepressant activities^{33 to 37}.

The Quantity of total phenolic and flavonoids of the water extracts of the selected medicinal plants is estimated using the colorimetric method. The results indicated that *T. tomentosa* stem bark has more phenolic and flavonoid contents. The Genus *Terminalia* belongs to the Combretaceae and comprises about 250 species. Of these, only 39 species were studied for its phytochemical composition and 368 phyto-constituents such as terpenoids, flavonoids, tannins, simple phenolics, phenylpropanoids, etc. Quantitative estimation of phenolic and flavonoid content from *T. tomentosa* stem bark was reported³⁸. They reported higher

values of TPC and less TFC than the contents of the present study. Budholiya and Sharma³⁹ estimated total phenolic and flavonoid contents from *T. tomentosa* leaf extracts.

Vasthi Kennedy and Devarajan Natarajan⁴⁰ studied antioxidant and phytochemical analysis of *A. alnifolia* leaf, especially methanol and aqueous extracts. In the present study, water extract has higher phenolic and flavonoid content than the previous report (Fig. 2A & B). TPC and TFC of *C. bonduc* seeds showed a lower range than previously reported results⁴¹. This may be due to the variation in the climatic condition in both atmospheric and edaphic conditions.

Phytochemical reports of the genus *Commiphora*, resulted in the identification of more than 300 chemical constituents⁴². Very few and sporadic attempts were made on phenolic and flavonoid contents of *C. caudata* leaf ethanol extract⁴³. *M. concanensis* a rare wild medicinal plant of tropical deciduous forest, received good attention in phytochemical reports of different parts. The results indicated the presence of volatile oils, flavonoids, alkaloids, tannins, and fatty acids⁴⁴. Few reports were noticed on quantitative analysis of phenolics and flavonoids from *M. concanensis*⁴⁵. A critical review of quantitative estimation of the phytochemical composition of aqueous extracts of *C. caudata* (stem bark), *C. spinarum* (fruits), and *M. concanensis* (leaves) revealed that no previous

report was noticed on total phenolic and flavonoid contents, hence, present report on the said species gains importance.

The standard and common method used to estimate the antioxidant capacity of medicinal plant extract is the phosphomolybdenum method. Antioxidant components reduce molybdenum and form a green coloured MO V complex. Revathi *et al.*,⁸ reported phosphomolybdenum dependent antioxidant capacity of *A. alnifolia* leaf extracts i.e., Petroleum ether (38.7 ± 2.2), methanol (139.7 ± 2.8) and hot water extract (82.9 ± 6.4 mg AAE/g). In our current study the total antioxidant capacity of water extract of *A. alnifolia* aerial parts showed similar values (80.13 ± 1.05 mg AAE/g) to the previously reported values. Based on the review of the antioxidant potential of aqueous extracts on ammonium molybdate dependent antioxidant capacity of *C. caudata* (stem bark), *C. spinarum* (fruits), *M. concanensis* (leaves) and *T. tomentosa* (stem bark) revealed that no previous report was noticed on ammonium molybdate dependent antioxidant capacity. Hence, the present report on the said species gains importance.

DPPH is a synthetic free radical commonly used to evaluate the antioxidant potential of herbal drugs/pure constituents. The results on DPPH reducing activity revealed that, among the test plant extracts, *A. alnifolia* strongly (88%) reduced DPPH purple color than other plant extracts. *T. tomentosa* exhibited the lowest IC₅₀ value (25 µg/ml), indicating its potential as an antioxidant. The intense DPPH quenching activity of *T. tomentosa* may be responsible for the presence of ellagic acid and gallic acid, which were reported as potential antioxidants/therapeutics from different traditional medicinal plant sources⁴⁶. Evangelene and Natarajan⁴⁷ observed the antioxidant activity of *A. alnifolia* leaf methanol and water extracts by the DPPH method. Here, we studied the DPPH scavenging activity of water extract of *A. alnifolia* aerial parts, the results demonstrate that water exhibited IC₅₀ values (42.5 µg/ml) higher than leaf extracts. DPPH reducing activity of *C. caudata* stem bark extracts ethyl acetate, methanol, petroleum ether and chloroform was reported⁴⁸. Here we reported DPPH quenching capacity of water extract. Vijay Kumar *et al.*,⁴⁹ reported DPPH scavenging activity of methanol extract of *M. concanensis* leaf. The results indicated that ethanol

and methanol extracts showed maximum inhibition of 69% and 66 % at 250 µg/ml concentration. In the present study, also reported the DPPH quenching capacity of water extract. It required 60 µg/ml to reduce 50% DPPH purple color. A critical review of the literature indicated that no previous report was noticed on the DPPH scavenging activity of water extract of *C. caudata* (stem bark), *C. spinarum* (fruits), *P. toemntosa* (leaves) and *T. toemntosa* (stem bark).

Hydroxyl radicals formed through Fenton's reaction are the most reactive oxidative molecules capable of damaging biological molecules/ membranes in living cells. In the present experiment, *T. tomentosa* exhibited maximum hydroxyl radical inhibition as ~96%. *A. alnifolia* showed the lowest IC₅₀ value (36.4 µg/ml), indicating its potentiality as hydroxyl radical inhibitor. Free radical scavenging property of *C. caudata* stem bark was reported by DPPH, nitric oxide, SOD methods by *in vitro*^{43, 48, 50} and *in-vivo* studies⁵¹. Antioxidant activity of leaf and fruit oils of *C. caudata* was reported by Reddy *et al.*,⁵². No previous report was noticed on the hydroxyl radical scavenging capacity of *C. caudata* stem bark extracts by *in vitro* studies.

The Hydroxyl radical quenching activity of *M. concanensis* leaf ethanol extract was studied by Balakrishnan & Krishnasamy⁵³. The results showed that, ethanol extract expressed very feeble activity i.e. it expressed a very high IC₅₀ value (400 µg/ml) and 70% as maximum inhibition at 500 µg/ml concentration. In the present study, water extract exhibited significant hydroxyl radical scavenging activity by expressing a low IC₅₀ value i.e., 60 µg/ml. Critical review on hydroxyl radical scavenging activity of *A. alnifolia* aerial parts⁵⁴, *C. spinarum* (fruits), *T. tomentosa* stem bark^{55, 56}, and *P. tomentosa* leaves⁵⁷ was not reported by earlier workers. Hence, the present work provides additional information on the hydroxyl radical scavenging potential of above mentioned plants.

CONCLUSION

The current *in vitro* studies indicated that water extracts of the selected medicinal plants have a high amount of phenolic and flavonoid components, good antioxidant capacity and

strongly inhibited Hydroxyl radicals and reduced DPPH, The findings of the study suggest that the selected medicinal plants could be used as a potential source of natural antioxidants.

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Conflict of Interest

Authors declared as no conflict of interest.

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