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*, *Comniphora 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), peroxyl 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 activities\(^3\). In this connection, we selected the following water extract of six medicinal plants to screen phytochemical analysis and antioxidant potential.

*Aclypha alnifolia* Klein ex Willd., is a rare medicinal plant found in the forests of South India\(^4\). *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 Lanka\(^9\). 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 properties\(^10\), the bark is a good remedy as anthelmintic and febrifuge and the leaves were reported as an emmenagogue\(^11\). 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 cancer\(^14\). 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*\(^9\). Different parts of this plant have been reported to treat different human ailments such as leaves for gynec problems, hyper-tention, 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 abortion\(^20\).

*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 countries\(^21\). In Ayurveda, the bark treats rheumatism, fever, urinary diseases and diabetes, vertigo, piles, constipation and chronic dysentery\(^22\).

**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 regional\(^23\) and local floras\(^24\).

**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 both. 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 methods\textsuperscript{25}.

Total phenolic content
Folin-Ciocalteu (FCA) reagent method was used to estimate the total phenolic content of the water extracts in the six medicinal plants\textsuperscript{26}. The detailed procedure was followed as described\textsuperscript{27}.

Total flavonoid content
Aluminum chloride method was used to estimate the total flavonoid content (TFC) of the water extracts of the six medicinal plants\textsuperscript{28}. The detailed procedure was followed as described\textsuperscript{27}.

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DPPH and Hydroxyl radical scavenging activity
DPPH and Hydroxyl radical activity of water extracts were measured as mentioned in detail elsewhere\textsuperscript{30, 31}. The detailed procedure was followed as described\textsuperscript{27}.

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

<table>
<thead>
<tr>
<th>Type of the Component</th>
<th>AA</th>
<th>CB</th>
<th>CS</th>
<th>CC</th>
<th>MC</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Catecholic compounds</td>
<td>T</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>T</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>T</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

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 ~80% as maximum inhibition. The extracts exhibited IC₅₀ values to inhibit 50% of the DPPH radical between 300 to 25 µg/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 IC₅₀ value (36.4 µg/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...
Plants synthesize a wide variety of chemical components such as alkaloids (nitrogenous compounds), terpenes (lipid derivatives) and phenolics (carbohydrate derivatives) with potential pharmacological properties. 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, betacitie, 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.

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 \textit{T. tomentosa} stem bark has more phenolic and flavonoid contents. The Genus \textit{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 \textit{T. tomentosa} stem bark was reported. They reported higher values of TPC and less TFC than the contents of the present study. Budholiya and Sharma estimated total phenolic and flavonoid contents from \textit{T. tomentosa} leaf extracts.

Vasthi Kennedy and Devarajan Natarajan studied antioxidant and phytochemical analysis of \textit{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 \textit{C. bonduc} seeds showed a lower range than previously reported results. This may be due to the variation in the climatic condition in both atmospheric and edaphic conditions.

Phytochemical reports of the genus \textit{Commiphora}, resulted in the identification of more than 300 chemical constituents. Very few and sporadic attempts were made on phenolic and flavonoid contents of \textit{C. caudata} leaf ethanol extract. \textit{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 acids. Few reports were noticed on quantitative analysis of phenolics and flavonoids from \textit{M. concanensis}. A critical review of quantitative estimation of the phytochemical composition of aqueous extracts of \textit{C. caudata} (stem bark), \textit{C. spinarum} (fruits), and \textit{M. concanensis} (leaves) revealed that no previous
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) and hot water extract exhibited significant hydroxyl radical scavenging activity by expressing a low 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 vitro43, 48, 50 and in vivo studies 51. 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 & Krishnasamy 53. 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 parts44, C. spinarum (fruits), T. tomentosa stem bark 55, 56, and P. tomentosa leaves 57 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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None.

Conflict of Interest

Authors declared as no conflict of interest.

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