Phytochemical Analysis, Total Phenolic Content, Flavonoids and Anti-oxidant activity of *Curcuma pseudomontana* rhizomes extracts

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Abstract:
Curcuma pseudomontana J Graham is one of the plant species reported from the Western Ghats of India, belonging to the family Zingiberaceae, with ethno botanical values. The present study is aimed to evaluate phytochemical analysis, total phenolic, flavonoid and Anti-oxidant activity of rhizomes extract of *Curcuma pseudomontana*. In the present research work, the rhizomes of *curcuma pseudomontana* powder were extracted by successive soxhlation extraction process with n-hexane, chloroform, ethyl acetate, and methanol and subjected to phytochemical analysis to find out various primary and secondary plant metabolites. The phenolic content and flavonoids was quantified by colorimetric method and antioxidant activity was determined based on the ability to scavenge free radicals by using DPPH assay and results are indicated that the presence of various phytochemical like Phenolic, Flavonoid , triterpenoids, alkaloids, tannins, saponins and carbohydrates are present in plant extracts. Among various extract methanol extract showed highest total Phenolic, flavonoid content and anti-oxidant activity compared to other extracts.
Key words: *Curcuma pseudomontana*, Phenolic Content, Flavonoids Content, Anti-oxidant activty

Introduction:

Medicinal plants have been used in several indigenous herbal practices since very old times to cure several diseases. Herbal medicines are still continues to serve as an important health care system even today despite the greater advancements in modern medicine systems in the recent years, their long uses in the folk medicine and their safer implications in human health have generated much interest in them, especially in developing countries. It has now been established that drugs derived from plant products are safer than their synthetic compounds[1].Plant and plant-based products are contains different phytochemicals such as phenols, flavonoids, alkaloids, glycosides, lignins, and tannins. Phenols and flavonoids are the most common phytoconstituents of different fruits, vegetables, medicinal and aromatic plants, which are responsible for antioxidant activities[2]. Due to the potential toxicological effects of synthetic antioxidants[3], natural antioxidants such as phenols and flavonoid compounds from plant origin are gaining popularity these days [4]. Hill turmeric (*Curcuma pseudomontana* Grah.) is an erect herb; it is a member of Zingiberaceae family and persistent native to the Western Gats in India and some of the South East Asia. Many centuries, hill turmeric has been used in Asia for its healing properties such as treatment of jaundice, body swelling, wound healing activities, liver ailments and blood

purification. Moreover, it has been used wide range of biological activities against diabetic, leprosy, inflammation, cancer and cardiac vascular diseases[5]. Dried rhizomes are used in skin diseases and impurities of blood [6.] Rhizomes boiled in oil and used as an application to sprain and useful on snake bite[7], rhizome powder are useful in leucoderma, scabies, smallpox, and intestinal worms as well as juice strong remedy against rheumatism and in combination of ginger used for smooth delivery in North East India[8] . Boiled tubers along with a pinch of salt in oral administration increase the secretion of milk among new mothers and lactating woman in Andhra Pradesh[9].The Bagata and Valmiki tribes of Munchingiputtu Mandal, Visakhapatnam district, Andhra Pradesh used in the treatment of jaundice and diabetes [10]. The rhizome are used for skin problems and coughs by the tribals of Achampet Forest Division in Nallamalais, Telengana, India [11],The Kattunaikan tribe of Malappuram district in Kerala, India, uses the rhizomes for cardiac disorders [12] . The rhizomes are used for muscle pain, leprosy and debility by tribal communities residing in Gundlabrahmeswaram Wildlife Sanctuary (Eastern Ghats), Andhra Pradesh, India[13].

Material and Method:

The fresh rhizomes are collected from the chikmagalur, Western Ghats of Karnatana, India and authenticated by Dr. Madhavashetty, Dept of Botanty; Sri Venkateshara University, Trupathi, Andhra Pradesh and the Voucher specimen was deposited in the herbarium of School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Medchal, Telengana.

Preparation of Extract:

Freshly collected rhizomes were dried at room temperature and coarsely powdered. The rhizomes powdered were extracted successively with n-hexane, chloroform, ethyl acetate and methanol using Soxhlet apparatus. The crude extract was evaporated to dryness and stored in airtight containers for future use. The obtained extracts were used for preliminary phytochemical screening by performing various chemical tests to detect the presence various phytoconstitients.

Phytochemical Screening:

The various phytochemical screening of plant extracts was done by following the standard methods[13,14, 15.]

Determined the total phenols content (TPC):

The total phenolic content was determined by using the Folin-Ciocalteu assay. An aliquot (1 ml) of extract or standard solution of gallic acid was added to a 25 ml volumetric flask, containing 9 ml of distilled water. A reagent blank was prepared using distilled water. One millilitre of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na2 CO3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 min at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV-Vis Spectrophotometer. Total phenolic content was expressed as mg gallic acid equivalents (GAE) [14].

Total flavonoids content (TFC):

Total flavonoid content was measured by the Aluminum chloride colorimetric assay. An aliquot (1 ml) of extracts and standard solutions of quercetin was added to a 10 ml volumetric flask containing 4 ml of distilled water. To the flask, 0.30 ml of 5% $NaNO_2$ was added and after 5 min, 0.3 ml of 10% $AlCl_3$ was added. After 5 min, 2 ml of 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE) [15].

Anti-oxidant Activity:

Assay for DPPH free radical scavenging activity:

The DPPH free-radical scavenging activity of *Curcuma pseudomontana* rhizomes extract was estimated as described earlier[16].Various concentrations of different extracts of *Curcuma pseudomontana* (0.5 ml each) were mixed thoroughly with 1ml methanol solution of 0.1 mM DPPH. The mixture was allowed to stand for 30 min in the dark. The absorbance was measured at 523 nm using a UV-VIS Spectrophotometer. An equal amount of DPPH and methanol were used as standard and blank, respectively.

The scavenging activity was calculated using the following formula:

Scavenging (%) = (A control – A sample)/A control × 100,

Where A sample is the absorbance of the test sample and A control is the absorbance of the control.

RESULTS:

The various phytochemical compounds present in extracts are reported in Table N0.1

Table 1: Preliminary phytochemical evaluation of *Curcuma pseudomontana* extracts

Phytochemical	Methanol	Ethyl acetate	Chloroform	N-Hexane
Alkaloids	+	-	+	-
Glycosides	+	-	-	-
Flavonoids	+	+	+	-
Tannins	+	-	+	-
Steroids	+	+	+	+
Tri terpenoids	+	+	+	+
Protein	+	+	+	+
Carbohydrates	+	-	+	-

+ indicate presene, - indicates absent

Methanolic extracts produced positively to all the tests for the presence of alkaloids, glycosides, flavonoids, tannins, steroids, triterpenoids, proteins and carbohydrates. The ethyl acetate extracts responded positively to the tests for flavonoids, steroids, triterpenoids and protein.The ethyl acetate extracts failed to respond to the test for alkaloids, glycosides, tannins and carbohydrates indicating their absence. Chloroform extracts given positive tests for proteins, alkaloids, Flavonoids, Tannins, steroids, Triterpenoids and proteins. These extracts were not found to

glycosides indicating their absence. The n-hexane extracts of the plant were found to respond positively to the presence of steroids, triterpenoids and Proteins. These extracts, however showed negative results to the tests for alkaloids, Glycosides, Flavonoids, Tannins, Phenolic and carbohydrates indicating their absence.

Total phenolic contents of different extracts plant

The result of total Phenolic contents of the various extracts are given in Table.2 Among these, methanol extract contained the highest (45.85 ± 0.26mgQE/g) amount of total flavonoid content compounds followed by ethyl acetate extract(35.55 ± 0.16),chloroform extract (30.75 ± 0.14mgQE/g) and n-Hexane extract shows (15.35± 0.08 mgQE/g).

Table.2 Total Phenolic content of *Curcuma pseudomontana* extracts

S.NO	Extracts	Total Phenolic Content (µg mL-1)
1	n-Hexane extract	15.35± 0.08
2	Chloroform extract	30.75 ± 0.14
3	Ethyl acetate extract	35.55 ± 0.16
4	Methanol extract	45.85 ± 0.26

Total Flavonoid contents of different extracts plant

The result of total flavonoid contents of the various extracts are given in Table 3. Among these, methanol extract contained the highest (19.65 ± 0.25mgQE/g) amount of total flavonoid content compounds followed by ethyl acetate extract (15.3 ± 0.14), chloroform extract (12.02 ± 0.12 mgQE/g) and n-hexane extract (14.4± 0.09 mgQE/g).

Table.3 Total Flavanoid content of *Curcuma pseudomontana* extract

S.NO	Extracts	Total Flavonoid Content (µg mL-1)
1	n-Hexane	14.4± 0.09
2	Chloroform fraction	18.20 ± 0.18
3	Ethyl acetate fraction	22.35 ± 0.24
4	Methanol extract	29.85 ± 0.66

Free radical scavenging activity by DPPH:

Table. 4: Effect of *Curcuma pseudomontana* extract on free radical scavenging activity

Extract	**25 µg/ml**	**50 µg/ml**	**75µg/ml**	**100 µg/ml**
Ascorbic acid	62.18±0.5	67.85±0.4	70.30±1.2	74.65±0.5
n-Hexane extract	22.33±0.4*	24.55±0.9*	25.33±0.6*	32.3±1.1*
Chloroform extract	32.66±0.5**	35.34±0.8**	40.66±0.7**	48.27±1.2**
Ethyl acetate extract	35.45±0.5**	39.66±1.1**	45.33±0.9**	51.63±1.1**
Methanol extract	40.33±0.4***	47.45±1.5***	55.32±1.4***	61.66±1.1***

Results expressed as the mean ± standard deviation (n = 3)

DPPH Radical Scavenging Activities of various extracts shows remarkable in vitro DPPH radical scavenging activities in a dose-dependent manner. The standard (L-ascorbic acid) exhibited significantly higher DPPH radical scavenging activities than the DPPH radical scavenging activities of all the studied plant extracts ($P < 0.05$). At all the studied concentrations, the methanolic extract produced significantly higher DPPH radical scavenging activities than other extracts.

Conclusion:

The present study revealed that methanol and ethyl acetate extracts of *Curcuma pseudomontana* is a good source of phytochemicals with antioxidant properties. Moreover, the methanol extract showed relatively high antioxidant activity. The phytoconstituents flavonoids, Phenolic compounds, tannins and saponins present in the extracts may be responsible for antioxidant activity and that the methanol extract of *Curcuma pseudomontana* extract possesses significant anti-oxidant activity.

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