International Journal of Drug Development & Research
| April-June 2013 | Vol. 5 | Issue 2 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
SJR Impact Value 0.13 & H index 2

Full Length Research Manuscript

Phytochemical screening and antioxidant activity of bark extracts of *Chionanthus zeylanica linn.*, as an important medicinal plant in eastern ghats

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Abstract

Chionanthus zeylanica Linn., belongs to the family Oleaceae. The stem bark part of *Chionanthus zeylanica* Linn., was dried, extracted with different solvents by soxhlet extraction method. Phytochemical studies of all the crude extracts showed the presence of secondary metabolites of flavonoids, steroids, tannins, alkaloids, glycosides, phenols, reducing sugars, etc. The phytochemical results confirm that all extracts contains more important chemical constituents for various biological activities. Antioxidant activity (DPPH) has been carried out for all the crude extracts of *Chionanthus zeylanica Linn.* The Ethyl acetate and methanol extracts have exhibited significant antioxidant activity in DPPH method. The results indicates that both the extracts firmly posses strong antioxidant effects. Comparatively the Ethyl acetate bark extract showed more antioxidant activity than the methanolic extracts. The results obtained from the present study indicates that the Chionanthus *zeylanica Linn.,* the plant bark extract can be potential source of natural antioxidant activity. This is the first report of antioxidant and phytochemical screening of all the crude extracts of the bark part of this plant.

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Key words:
Chionanthus zeylanica Linn., DPPH method, Antioxidant activity, phytochemical screening, soxhlet extraction,

How to Cite this Paper:
Venkateswara Rao. S, Ayya Raju. M, Kammakshamma. J* "Phytochemical screening and antioxidant activity of bark extracts of Chionanthus zeylanica linn., as an important medicinal plant in eastern ghats" Int. J. Drug Dev. & Res., April-June 2013, 5(2): 311-316.

Article History:----------
Date of Submission: 10-04-2013
Date of Acceptance: 26-04-2013
Conflict of Interest: NIL
Source of Support: NONE

INTRODUCTION:

Phytochemicals are chemical compounds for the duration of the plants usual metabolic processes. These chemicals are regularly referred to as

"secondary metabolites" of which there are numerous classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids.[1-2] According to world health organization (WHO) more than 80% of the Asian and African population relies on medicinal plants based research in their primary health care needs.[3] Numerous phytochemical surveys have been available, including the random examples approach which involved various plant accessing collected from every part of the world. The most important chemical substances of concentration in these surveys were the alkaloids and steroidal sapogenins, however other diverse groups of naturally occurring phyto-components such as flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils etc., have also been reported.[4] The medicinal importance of plants play role in a some chemical substances that create a specific physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno-pharmacological information is commonly measured an effective approach in the discovery of new anti-infective agents from higher plants.[5] It is normally established that in a condition of oxidative stress, Reactive Oxygen Species (ROS) such as superoxide (O_2), hydroxyl (OH^-) and peroxyl radicals are generated. The ROS play a main role in the pathogenesis of different serious diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts, and inflammation.[6-8] Antioxidant compounds in food play a main role as a health caring factor. Scientific support suggests that antioxidants decrease the danger for chronic diseases including cancer and heart diseases.[9-11].

The responsibility of free radicals and tissue damage in diseases such as atherosclerosis, heart failure, neurodegenerative disorders, ageing, cancer, diabetes mellitus, hypertension and numerous other diseases are becoming increasingly accepted. Antioxidant supplements or food rich in medicinal plants may be used to help the human body in falling oxidative damage by free radicals and active oxygen.[12] Systematic indigenous knowledge gathering on the utilization of known plants among communities may result in the discovery of novel and effective compounds and plant derived commercial food products.[13-14] Therefore presently, the research importance is focused on the potential role of antioxidant in the action and prevention of above diseases. The most commonly used antioxidants at present are Butylated hydroxyl anisole (BHA), Butylated hydroxyl toluene (BHT), Propyl Gallate (PG) and Tert-Butyl Hydro Quinone (TBHQ), Ascorbic acid (vitamin-c). However, they are suspected of being dependable for liver damage and carcinogenesis in laboratory animals.[15] Therefore, the growth and use of more helpful antioxidants of natural origin are most wanted. Now a day's most popular diseases(diabetes, cancer).Increased intracellular generation of ROS (Reactive Oxygen Species) has been proposed as a mechanism of tissue injury associated with a variety of pathological manifestations like diabetes, cancer, neurodegenerative disease, inflammation, atherosclerosis and thrombosis.[16] The most important difficulty is that H_2O_2 simply crosses cellular membranes while receiving one more electron, generally originating from iron or copper, gives rise to the hydroxyl radical. This radical represents the most reactive oxygen species, as it wants only one more electron to be stabilized. Cells living under aerobic environment continually face the oxygen (O2) paradox: O2 is essential to maintain life, but its metabolites such as reactive oxygen species (ROS) can change cell functions, endanger cell survival, or both.[17] Flavonoids are effective water-soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anti-cancer activity and protects in opposition to all stage of carcinogens. Flavonoids in the body are known to reduce the risk of heart diseases.[18]

PLANT TAXONOMICAL DESCRIPTION:

Chionanthus zeylanica L., (FIG 1) belongs to the family Oleaceace Sp. Pl. 1753; Bhargavan in Fl. Tamil Nadu 2:70. 1987. *Linociera zeylanica* (L.) Gamble in Fl. Pres. Madras 2:558. 1957 (repr. ed.); FTC 3:890. L. Purpurea Vahl, Enum. Pl. 1:47. 1804; FBI 3:608. Evergreen shrubs or small trees, 2-5 m tall. Leaves 3-7 x 2-4 cm, ovate-obovate, obtusely acute or emrginate, base cuneate, thick coriaceous, glaucous green. Flowers 4-5 mm across, white mildly fragrant in 3-5 cm long; axillary panicles. Drupes 4-6 x 2-3 mm, ovoid-oblong, apex mucronte, purplish-brown. Common in dense scrubs from coast to foot of hills. Fl & Fr: January-May. Ln: Punagani, Punisi. Lu: Bark used to control white discharge in ladies. Ld: Penchalakona: ASR 8611: Sriharikota : ASR 417: Venkatagiri: ASR 2607. World: Sri Lanka, Peninsular India.[19-20] Commonly grow in dry deciduous forests. Papanasanam, Akkagaralagudi, Microwave station and Srivariparvetimandapam area in Tirumala, Chelluru reserve forest, Mamandur, Talakona. Kilasakona.[21] The present paper deals with the phytochemical screening and antioxidant activity for all crude extracts of the plant material.

FIG 1: *Chionanthus zeylanica* *Linn.,* FRIUTING AND STEM BARK

MATERIAL AND METHODS:

PLANT MATERIAL:

C. zeylanica *L.* was collected freshly from Eastern ghats of Seshachala hills of Chittoor district. Andhra Pradesh, India. The plant was identified and authenticated by Head of the department, Botany and the voucher specimen No. ASR 2607 was deposited in the Department of Botany, Sri Visovodaya Government Degree college (Botany Research center) Venkatagiri, Nellore District, Andhara Pradesh. The bark part of ***C.zeylanica*** L. was washed with distilled water, shade dried, powdered, and stored in and air tight container for further use.

PREPARATION OF PLANT EXTRACTS:

About 500g of the air dried powder of the plant material ***C. zeylanica*** *L.* was extracted successively with the following solvent in soxhlet extractor, and identified as fractions 1-3 as shown below; n-hexane-Fraction-1, Ethylacetate-Fraction-2, and Methonal-fraction-3. Every time before extracting with the next solvent, the plant material was dried in hot air oven below 50^{0} C. Each extract is concentrated by distilling off the solvent and then evaporating to dryness. The obtained extracts were subjected to qualitative test for the identification of various phyto- constituents.

PHYTOCHEMICAL ANALYSIS OF THE PLANT EXTRACT:

PRELIMINARY SCREENING TESTS FOR SECONDARY METBOLITES:

Preliminary phytochemical analysis of the Hexane, Ethyl acetate and methanolic crude extract were carried out to detect the various classes of secondary metabolites in the selected plant materials by adopting standard qualitative methods as described.[22-26] Qualitative screening of phytochemicals from *C.zeylanica.L.*is shown in Table-1.

ANTIOXIDANT ABILITY ANALYSIS:

DPPH (1, 1- diphenyl-2-picrylhydrazyl) solution (0.1mM) was prepared in methanol by dissolving 1.4 mg of DPPH in methanol and the remaining volume was made 100ml with Methanol. The solution was kept in darkness for 30 minutes to complete the reaction. The free radical scavenging activity of the *C. zeylanica* extracts was determined by DPPH. This antioxidant activity was measured by following method described.[27] Briefly, 1ml of 0.1mM methanolic DPPH solution was added to 3ml of different plant crude extracts, at different concentrations (50, 100, 150, 200 µg/ml). The mixture was vigorously shaken and left to stand for 30 minutes under subdued light. The absorbance was measured at 517 nm in a UV spectrophotometer. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid (Vit-C) and BHT which are good antioxidants, was taken as a standard in this study. The DPPH radical scavenging activity was calculated by using the following equation:

DPPH Scavenging activity (%) = (1-As/Ac) ×100

Where, As: Absorbance of the sample.

Ac: Absorbance of the control.

STATISTICAL ANALYSIS:

Antioxidant analysis was expressed as means (±) standard deviation of (6n) measurements. One-way analysis of variance (ANOVA) was applied using (SPSS v. 15) in order to detect significant differences in 6 extract mentioned in Table-2.

RESULTS AND DISCUSSION:

PHYTOCHEMICAL ANALYSIS:

Table-1. shows that the results of the qualitative screening of phytochemical analysis from *C. zeylanica Linn*. Flavonoids, steroids, terpeniods, tannins, glycosides, alkaloids and reducing sugars are present in Ethyl acetate, methanolic extracts. But Phenols, Quinones, Lignin, fixed oils are present in methanol extract only. These phytochemical compounds are known to support bioactivity. Thus responsible for the antioxidant activities.

Table 1- Qualitative screening of phytochemicals from the plant of *C. zeylanica L.*

Phyto constituents	n-hexane	Ethyl acetate	Methanol
Flavonoids	-	+	+
Steroids	+	+	+
Terpenoids	-	+	+
Tannins	-	+	+
Glycosides	+	+	+
Soponins	_	_	_
Alkaloids	+	+	+
Phenols	_	_	+
Quinones	_	_	+
Lignin	_	_	+
Fixed oils	_	_	+
Reducing sugars	_	+	+

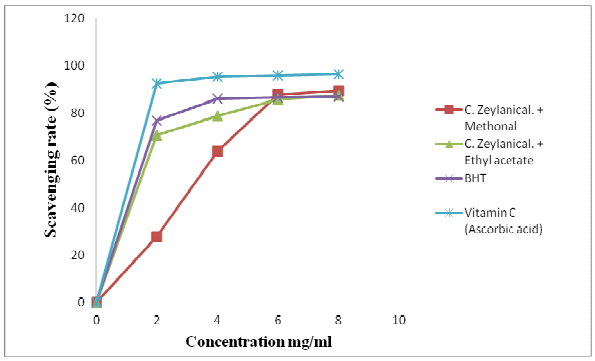
DPPH FREE RADICAL SCAVENGING ACTIVITY:

Table-2. shows the result of the DPPH free radical scavenging activity of the enzymatic extracts in *C. zeylanica L* (Linn). The highest DPPH radical scavenging activity recorded was 37.7± 0.2 at the concentration of 200µg/ml. reports that antioxidant activities of bark extracts in *Chionanthus zeylanica L* (Linn). In our experiment, there is a non-enzymatic antioxidant i.e. ascorbic acid and BHT which were taken as standard. No report was found regarding antioxidant activity of *C. zeylanica L*. Few research studies have been undertaken on the antioxidant activity of enzymes in medicinal plants using DPPH scavenging assay (FIG-2)**.**[28-30]

Table 2: Various crude extracts of *C.zeylanica* for antioxidant activity

	C.zeylanica Methanol extract	C. zeylanica Ethyl acetate	BHT	Vit- C
Control	0.293 ±0.00237	0.293 ±0.00237	0.293 ±0.00237	0.293 ±0.00237
50 µl	0.2128 ±0.00232	0.0862 ±0.00133	0.0685 ±0.00187	0.222 ±0.00133
100 µl	0.1065 ±0.00288	0.0622 ±0.00133	0.0417 ±0.00197	0.0147 ±0.00216
150 µl	0.0367 ±0.00288	0.0427 ±0.00121	0.0398 ±0.00160	0.0123 ±0.00186
200 µl	0.0313 ±0.00234	0.0375 ±0.00217	0.387 ±0.00273	0.0105 ±0.00138
F-value	11873.821	1206.615	281.098	53.427

Fig 2: SCAVENGING RATE (%) OF PLANT EXTRACTS AND KNOWN ANTIOXIDANTS:



CONCLUSION:

The present study suggests that qualitative phytochemical screening of crude extracts of *C.zeylanica* L. supports the presence of bioactive compounds such as Flavonoids, steroids, terpeniods, tannins, glycosides, alkaloids reducing sugars and Phenols, Quinones, Lignin and fixed oils in the medicinal plant and thus responsible for the antioxidant activities. Bark extracts possesses potent antioxidant activity. Therefore could be a potential source of natural antioxidant that could have great importance as therapeutic agent in preventing or slowing down the progress of ageing and age associated oxidative stress related degenerative diseases. Further research is recommended for better characterization of important bioactive constituents responsible for antioxidant activity. The revealed antioxidant property of extracts may provide potential therapeutic intervention against oxidative threats and degenerative disorders.

ACKONWLEDGEMENT:

Grateful thanks to UGC New Delhi (Major Research Project) for providing financial support for the completion of this work.

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