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Evaluation of Brine Shrimp Lethality of *Cinnamomum* Species

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ABSTRACT

Cinnamomum species have long been used as spices. The preliminary bioactive constituent's identification and brine shrimp lethality activities of ethanolic extracts of seven *Cinnamomum* species viz., *C. travancoricum*, *C. walaiwarense*, *C. wightii*, *C. verum*, *C. sulphuratum*, *C. riparium*, and *C. perrottetii* were evaluated in this study. The results of cytotoxic activity of the bark extracts of seven *Cinnamomum* species were more active than leaf extracts against brine shrimp lethality of *Artemia salina*.

Keywords: *Cinnamomum* species; bioactive constituents; Brine shrimp; *Artemia salina*; cytotoxic activity.

INTRODUCTION

In the United States in 1999, over 1500 people are expected to die of cancer each day, representing an estimated total mortality rate of about 560000. More than twice as many persons than this will be diagnosed with invasive cancer, but, overall, a slight decline in cancer incidence rates has been observed in the USA. Among many recent advances in cancer chemotherapy, phytochemicals play an important role in cancer chemotherapeutic drugs. A search for new anti-cancer drugs has taken many different approaches. The brine shrimp lethality bioassay is efficient, rapid and inexpensive tests that require only a relatively small amount samples. The technique is easily mastered, costs little, and utilizes small amount of test material. Meyer et al.(1982) has been successively employed for *in-vivo* lethality bioassay-guide fractionation of active cytotoxic and antitumor agents such as trilobacin from the bark of *Asimina triloba* (Zhao et al., 1992), *cis*-annonacin from *Annona muricata* (Rieser et al. 1996) and ent-kaur-16-en-19-oic acid from *Elaeoselinum foetidum* (Mongelli et al. 2002).

The genus *Cinnamomum* belongs to the family Lauraceae and comprises several hundred species found in continental Asia, East and Southeast Asia, Australia, the Pacific, and a few species in Central and South America (Jantan et al.,1995). These are evergreen trees and shrubs and most of the species are aromatic. Twenty-one species of Cinnamomum have been found on the Malaysian peninsula (Kochummen, 1989). A number of these species are used in traditional medicine and their distilled essential oils or synthetic analogs are used as flavoring agents in the food and beverage industry (Burkill,1966; Jham *et al.*, 2005). Previously, brine shrimp lethality activity has not been evaluated seven *Cinnamomum* species. The present study was work carried out the preliminary phytochemicals identification and cytotoxicity properties of both barks and leaf extract on seven *Cinnamomum* species.

MATERIALS AND METHODS

Collection of Plant materials.

Seven *Cinnamomum* species (*C. travancoricum*, *C. walaiwarense*, *C. wightii*, *C. verum*, *C. sulphuratum*, *C. riparium*, and *C. perrottetii*) were collected from Kalakad Mundanthurai Tiger Reserve Forest, Karaiyar, Tirunelveli District, South India.

Extraction.

Dried 500gms of leaves powdered were individually extracted in a Soxhlet apparatus with ethanol (1.5lrs) at 60^{o}C for 8hr. Collected extracts were then filtered and concentrated in *vacuo* at 45^{o}C. The ethanol free extracts were kept refrigerated (4^{o}C) and used for the further investigations. Done on preliminary phytochemical identification methods followed by Maridass, (2005).

Brine shrimp lethality assay.

In vitro lethality assay of *A. salina* was used to detect cell toxicity (Meyer et al. 1982). Brine shrimp eggs were placed in seawater (3.8% w/v sea salt in distilled water) and incubated at 24-28^{o}C in front of a lamp. Eggs were hatched within 48h providing large number of larvae (nauplii). A convenient number of nauplii were placed in vials containing 5ml of seawater and increasing concentrations of Cinnamomum species viz., *C. travancoricum, C. walaiwarense, C. wightii, C. verum, C. sulphuratum, C. riparium,* and *C. perrottetii* leaves extract (0.1-500ppm). Control was made with the same volume of 96% ethanol in seawater without addition of the *Cinnamomum* species leaves extract. Alive nauplii were counted after 16h and the lethal Concentration (LC_{50}) was calculated.

Statistical analysis.

Lethality assays were evaluated by Finney computer statistical program to determine the LC_{50} values and 95% confidence intervals. All other data were expressed as mean ± SD.

RESULTS AND DISCUSSION

The cytotoxic activity of both barks and leaves of seven *Cinnnamomum* species were investigated *in vitro* tested against the brine shrimp (*Artemia salina*). The results are given in Table 1. All the crude extracts of *Cinnamomum* species resulting in LC_{50} values of less than 250µg/ml were considered for active against brine shrimp. This bioassay has a good correlation with cytotoxic activity in several plant extracts of *Ocimum sanctum*, *Lagerstroemia reginae*, *Cissampelos pareira*, *Acacia conccina*, *Punica granatum*, *Aconitum species*, *Rosa damascene*, *Cinchona species*, *Bacopa monnieri*, *Symplocos racemosa* were showed significant lethality to brine shrimp(Krishnaraju et al. (2006). In less active against brine shrimp lethality effect of ehtanolic extract of *Annona crassiflora* leaves are earlier reported (Pimenta et al. 2003). In the present reports of both plant parts of bark and leaf of all the *Cinnamomum* species showed good brine shrimp lethality. In bioactive constituents identification of seven *Cinnamomum* species with cytotoxic properties, activity due to the presence of triterpenes, fixed oils, sugars, saponins and tannins were observed (see Table 2). Alkaloids were absent in both leaves and barks of *Cinnamomum* species but all the plants samples gave positive reaction of both Liberman-Burchard and Salkowski tests indicating the presence of triterpenoids. The ferric chloride test gave positive results indicating the presence of catechol tannins in both parts of leaves and barks of all *Cinnamomum* species. Previously, strong reactions froth test were observed saponins compound present in the leaf of *Gomphandra lysipetala*, *Maesa ramentacea*, *Alphitonia excelsa*, and *Kleinhovia hospital* (Lailay, 2002). The present results agree with the presence of terpenoids, essential oils, fixed oils, saponins and tannin may be acte constituents useful for the utilization of cytotoxic principles of these extracts of *Cinnamomum* species. Further studies should be going on fractionation and identification of bioactive constituent to human cell line culture of cytotoxic effect.

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Table 1. Brine shrimp bioassay results of leaves extract of *Cinnamomum* species.

Plants	**Parts**	**LC_{50} µg/ml**
C. travancoricum	Leaf	226.5

	Bark	112.7
C. walaiwarense	Leaf	156.0
	Bark	148.2
C. wightii	Leaf	256.4
	Bark	125.1
C. sulphuratum	Leaf	132.4
	Bark	112.0
C. riparium	Leaf	112.3
	Bark	96
C. perrottetii	Leaf	189.0
	Bark	156.9
C. verum	Leaf	196
	Bark	75.5
C. glanduliferum	Leaf	87
	Bark	110.3
C. glaucescens	Leaf	136.5
	Bark	85

LC_{50}'s were estimated using logit transformation. Therefore confidence intervals are not provided.

An average of three replicates.

Table 2. Preliminary phytochemicals identification of *Cinnamomum* species.

Plants	Parts	Presents of phytochemicals							
		Alkaloids	Sugar	Triterpenoids	Flavonoids	Essential oils	Fixed oils	Sapanin	Tannins
C. travancoricum	Leaf	-	+	+	-	+	-	+	+
	Bark	-	+	+	-	+	+	+	+
C. walaiwarense	Leaf	-	+	+	-	+	-	+	+
	Bark	-	+	+	-	+	+	+	+
C. wightii	Leaf	-	+	+	-	+	-	+	+
	Bark	-	+	+	-	+	+	+	+
C. sulphuratum	Leaf	-	+	+	-	+	-	+	+
	Bark	-	+	+	-	+	+	+	+
C. riparium	Leaf	-	+	+	-	+	-	+	+
	Bark	-	+	+	-	+	+	+	+
C. perrottetii	Leaf	-	+	+	-	+	-	+	+
	Bark	-	+	+	-	+	+	+	+
C. verum	Leaf	-	+	+	-	+	-	+	+
	Bark	-	+	+	-	+	+	+	+
C. glanduliferum	Leaf	-	+	+	-	+	-	+	+
	Bark	-	+	+	-	+	+	+	+
C. glaucescens	Leaf	-	+	+	-	+	-	+	+
	Bark	-	+	+	-	+	+	+	+

“+” Presence of bioactive compound “-” Absence of bioactive compound