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PHYTOPHARMACOLOGICAL PROFILE AND BRINE SHRIMP LETHALITY ASSAY OF THE METHANOLIC AND ETHANOLIC EXTRACTS OF THE LEAF AND BARK OF *SYMPLOCOS COCHINCHINENSIS.*

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ABSTRACT

Cancer is one of the deadliest diseases in the world. Researchers all over the world are in constant search for new drugs to cure cancer. Traditional medicinal practices have utilized various plant sources for the treatment of cancer. Medical scientists all over the world have started exploring the traditional medicinal practices to prepare new formulations to treat cancer. The present study is one such approach. The cytotoxicity of the methanolic and ethanolic extracts of the leaf and bark powder of *Symplocos cochinchinensis* against brine shrimp larvae is done for the first time. It was found that the ethanolic extracts of the leaf showed 100% mortality at 2 mg/ml concentration in the 24th hour. The methanolic and ethanolic extracts of the bark showed 100% mortality at 3 mg/ml concentration in the 24th hour. The methanolic and ethanolic extracts of *Symplocos cochinchinensis* showed the presence of various phytoconstituents like phenols, flavonoids, tannins, saponins and glycosides. The methanolic extract of the leaf has the maximum phytoconstituents viz. Total phenolic content of 13.67$\pm$0.29mgTAE/g dry weight of the plant material., Total flavonoid content 6.34$\pm$0.4 µgQE/g dry weight of the plant material.. The free radical scavenging activity of the extracts was done by *Ferric* Thiocyanate (FTC), *Ferric reducing antioxidant power (*FRAP), *Thiobarbituric acid (*TBA) and *[2,2– -Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] (*ABTS) assays. The methanolic extract of the leaf showed the maximum inhibition in the FTC method (84.54%) and TBA (88.89%) assays. Methanolic extract of the bark has the maximum ability to reduce ferric ions (73.74%) as revealed by the FRAP assay. The ethanolic extract of the leaf was found to scavenge the ABTS radicals to a higher extent (55.71% in ABTS assay). In the present study the methanol and ethanol extracts of the leaf and bark of *S.cochinchinensis* was found to have free radical scavenging activity as shown by the *2, 2 – Diphenyl-1-picryl hydrazyl (*DPPH) assay.The phytopharmacological profile of the two extracts of the leaf and bark of *S.cochinchinensis* are also analysed. This study proves that the methanolic and *ethanolic* extracts of the leaf and bark of *S.cochinchinensis* are potential candidates for further research.

KEYWORDS: ***Symplocos cochinchinensis, antioxidant, DPPH, brine shrimp, cytotoxicity, phytoconstituents.***

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INTRODUCTION

Cancer is one of the deadliest diseases worldwide. Every year, millions of people are diagnosed with various types of cancer, millions die and millions are under treatment. Cancer still remains to be an aggressive killer. The International agency for Research on Cancer estimates of the incidence of mortality and the prevalence of major types of cancer, at national level, for 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer worldwide. 1(By 2030, it is projected that there will be 26 million new cancer cases and 17 million cancer deaths per year.The development of novel synthetic drugs or novel drug delivery systems has not been successful in the curing of cancer. So, much focus is on the development of new, effective and affordable anticancer drugs [3]. Natural products have received increasing attention over the past 30 years for their potential as novel cancer preventive and therapeutic agents [4,5]The favorite source is the compounds from the medicinal plants. There are millions of medicinal plants worldwide, which are unexplored till date. Many of these plants may contain a number of active compounds which can be potential drugs against cancer. Tannins are naturally occurring water soluble phenolic compounds [6] A review of tannins and human health had been carried out by Chung etal. [7]. Condensed tannins of higher molecular weight are commonly described as phlobatannins [8]. They are formed due to the aging of tissues [9]or due to enzymatic action on dead cells [10]Flavonoids and phenolics are the most important group of secondary metabolites and bioactive compounds in plants[11].They possess diverse biological activities such as anti ulcer and anti inflammatory [12] and as an anti oxidant[13]. Antidiabetic activity and anti cancer activity of flavonoids have been reviewed[14,15]Bioactive compounds are often toxic to shrimp larvae. Hence, invitro lethality to shrimp larvae can be used as a rapid and simple preliminary monitor for bioactive compounds during the isolation of natural products. Cytotoxicity to brine shrimp, *Artemia salina*, larvae is a rapid, inexpensive, in house, general bioassay which has been developed for screening, fractionation and monitoring of physiologically active natural products.[16] Plant species belonging to many genera have medicinal properties.[17] One such genus is *Symplocos.* This genus is widespread all over the world, but only a very few species of this genus have been extensively studied for their medicinal properties. A species little explored in this genus is *Symplocos cochinchinensis* which is distributed in tropical and sub tropical Asia. It is a small evergreen tree reaching a height upto 7 m with thin, smooth, light grey bark and white wood.[18]*S.cochinchinensis* has many uses in the indigenous system of medicine. The bark is astringent, acrid, ophthalmic, expectorant, anti inflammatory, depurative, febrifuge, haemostatic and stomachic. According to the Ayurveda system of medicine, it is useful in vitiated conditions of pitta and kapha, asthma, bronchitis, dropsy, arthritis, ulcers, leprosy, skin diseases, ulmeorrhagia, dyspepsia and gonorrhea[19]. Its bark is described as bitter and pungent which is used as an aphrodisiac and in menorrhagia, the diseases of "raktpitta" and against the disease of the eyes.[20] Bark is used in ophtahalmia and in threatened abortion.[21]The "Sarabendra vaidya muraigal" (a text generated by many ayurvedic, siddha and unani physicians at the period of King Sarfoji II) reports the use of *Symplocos cochichinensis* (Lour.)S.Moore. to treat diabetes mellitus.[22,23] Ved[24]reported the use of three species of *Symplocos* viz *Symplocos racemosa, Symplocos paniculata* and *Symplocos cochinchinensis* as *"lodhra"* for treating diabetes mellitus. The decoction of the leaves is valued in Indian medicines to treat diabetes. Paste of the leaves, boiled in oil is used for application in the scalp diseases.[21] The leaves impart a yellow dye which is used as a mordant. The fruits and seeds are strung into rosaries.[21] The wood is white, soft and even grained. It is used for making temporary rafts [21] and as fuel 26 and is used for match splints.[21]

MATERIALS AND METHODS

Plant source and preparation of the plant extracts

The leaves and barks of *Symplocos cochinchinensis* (Figures 1,2) were collected from the Western Ghats, Nilgiris, India in the month of June 2015. The plant parts were authenticated by Dr.Chelladurai, Research officer, Central council for research in Ayurveda and Sidda. The leaves and bark were a shade dried and powdered. The methanolic and ethanolic extracts of the dried powders were prepared. 10 g of the dried powder was dissolved in 100 ml of methanol and 1:2 ratio of ethanol – water. The contents were stirred well and left for 48 hours at room temperature. The filtrate collected after cold percolation was used for further analysis.

Phytochemical analysis

The methanolic and ethanolic extracts obtained from the dried leaves and bark powder of *Symplocos cochinchinensis* were tested for the presence of phytochemicals – Tannins, Phlobatannins, Flavonoids, Terpenoids, Cardiac glycosides and Steroids following the methodology described by Evans.[8]

Tests for Tannins

To 5 ml of the extracts, a few drops of 0.1% of Ferric chloride were added. The presence of brownish green or blue black colour indicated that the plant material possessed Tannins.

Tests for Phlobatannins

10 ml of the plant extracts was boiled with 1% HCl in a test tube. The presence of Phlobatannins was confirmed by the deposition of red precipitate in the test tube.

Tests for Saponins

To 10 ml of the extracts, 3 ml of distilled water was added and shaken well, so as to obtain froth. To the froth formed, a few drops of olive oil were added. The

formation of emulsion indicates the presence of saponins.

Test for Flavonoids

A few drops of 1% liquid ammonia were taken in test tubes, to which the methanolic and ethanolic extracts were added. Yellow colouration of the solution confirmed the presence of Flavonoids.

Test for Terpenoids

Around 2 ml of chloroform and 3 ml of concentrated sulphuric acid were added consecutively to 5 ml of the plant extracts. A reddish brown interface in the solution denoted the presence of Terpenoids.

Test for Cardiac Glycosides

To 5 ml of the plant extracts, 2 ml of glacial acetic acid containing a drop of Ferric chloride was added. This was followed by the addition of 1 ml of concentrated Sulphuric acid. The brown ring thus obtained yield positive result for the test.

Test for Steroids

A couple of grams of plant powder were mixed with 10 ml of chloroform followed by boiling and filtration. To the above 2 ml of the filtrate 2 ml of acetic anhydride and a few drops of concentrated sulphuric acid were added. Stable presence of blue green ring in the solution confirms the presence of steroids.

Determination of Total phenolic content

Folin- ciocalteau method was followed for the determination of the total phenolic content. Distilled water (500µl) and Folin –ciocalteau reagent (500µl) were added to 100 µl of the plant extracts and incubated at room temperature for 6 minutes. The final volume was made up to 3 ml with 7% sodium carbonate solution. The absorbance was measured at 760 nm using UV-visible spectrophotometer after an incubation period of 90 min. The total phenolic content was expressed as milligrams of tannic acid equivalents per gram of dry weight (mg TAE/gDW) of the plant using a standard plot of tannic acid.[9]

Determination of Total flavonoid content

The total flavonoid content of the plant was determined by the method adopted by Moussa *et al*. (2011). The plant extracts were taken in test tubes and the solvent were allowed to evaporate. To the residue 5 ml of 0. 1 M Aluminum chloride was added and shaken well. This was followed by incubation for 40 minutes at room temperature and the absorbance value was measured at 415 nm using UV -Vis spectrophotometer. A standard plot of quercetin at varying concentration was used to evaluate the total flavonoid content, expressed as milligrams of Quercetin equivalent per gram dry weight (mg QE/gDW) of the plant material[10].

Determination of the Total antioxidant activity

The total antioxidant activity was estimated by phosphomolybdenum method. To the plant extract (0.5 ml), 4.5 ml of the reagent solution (0.6 M sulphuric acid, 28 mm sodium phosphate and 4 mM of ammonium molybdate) was added. The solution was maintained in a boiling water bath at 95 C for 90 min. The solution was cooled to room temperature and the absorbance was measured at 695 nm using UV- visible spectrophotometer. The total antioxidants in the plant were expressed as mg TAE/g DW of the plant material.[11]

2, 2 – Diphenyl-1- picryl hydrazyl (DPPH) free radical scavenging assay

The two plant extracts were taken at various concentrations (10,20,30,40,50 µg/ml) in small test tubes and made up to 1 ml using methanol. 1 ml of 0.01 mM DPPH dissolved in methanol was added to all the test tubes and maintained in dark for 30 minutes, at room temperature. The absorbance of the solutions was read at 517 nm. The percentage inhibition and IC 50 values were calculated with DPPH as the control and butylated hydroxyl anisole (BHA) as the reference. The concentration in µg of dry material per ml of solvent (µg/ml) that inhibits the formation of DPPH radicals by 50% is defined as the IC 50 value.[12]

$$\% \text{ inhibition} = \frac{(\text{absorbance of control (Ac)} - \text{Absorbance of the sample (As)}) \times 100}{\text{Absorbance of the control (Ac)}}$$

***Ferric* thiocyanate (FTC) assay**

The assay involves the addition of 120 µl of 98% ethanol, 100 µl of 2.5 % linoleic acid and 9 ml of 40 mM phosphate buffer (pH 7) to 100 µl of the plant extract. To 100 µl of the mixture, 9.7 ml of 75% ethanol, 100 µl of 30% ammonium thiocyanate and 100 µl of 20 mM $FeCl_3$ in 3.5% HCl were added after maintaining the solution in the dark, at 40 ° C. The absorbance of the solution was measured at 500 nm using UV-visible spectrophotometer after 3 min. The percentage of inhibition was calculated with Tannic acid as the standard.[13]

$$\% \text{ inhibition} = \frac{(\text{absorbance of control (Ac)} - \text{Absorbance of the sample (As)}) \times 100}{\text{Absorbance of the control (Ac)}}$$

Thiobarbituric acid (TBA) assay

Equal volume (200 µl) of 20% trichloroacetic acid and 0.67% thiobarbituric acid were mixed with 1 ml of 2.51% linoleic acid and 1 ml of plant extract. The solution was maintained in boiling water bath for 10 min; cooled to room temperature and centrifuged at 3000 rpm. The supernatant was subjected to UV–visible spectrophotometric analysis at 532 nm. The percentage inhibition of the plant against the secondary products of lipid peroxidation was evaluated with reference to the standard solution of butylated hydroxyl toluene (BHT).[13]

$$\% \text{ inhibition} = \frac{(\text{absorbance of control (Ac)} - \text{Absorbance of the sample (As)}) \times 100}{\text{Absorbance of the control (Ac)}}$$

Ferric reducing antioxidant power (FRAP) assay

1 ml of plant extract, 2.5 ml phosphate buffer (of 0.2 M, pH 7) and 1% potassium ferricyanide (2.5 ml) were mixed and incubated at 50 ° C for 30 min. To the solution, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 6500 rpm for 10 min. Distilled water (2.5 ml) and 0.5 ml of 0.1% $FeCl_3$ were added to 2.5 ml of the supernatant. The absorbance of the solution was measured at 700 nm using UV–visible spectrophotometer. The reducing ability of the plant was evaluated in terms of percentage by relating to the standard, $FeSO_4$. [14].

$$\% \text{ inhibition} = \frac{(\text{absorbance of control (Ac)} - \text{Absorbance of the sample (As)}) \times 100}{\text{Absorbance of the control (Ac)}}$$

[2,2–-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] ABTS assay

A solution of 7 mM ABTS [2,2 -azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] and 2.45 mM potassium persulphate was incubated in the dark for 12–16 h, after which the solution was diluted with ethanol till the absorbance reached 0.7 ± 0.02 at 734 nm. 1 ml of the diluted solution was mixed with 100 µl of plant extract and the absorbance was evaluated at 734 nm after 6 min. The percentage reduction against ABTS was calculated with reference to the standard, Tannic acid.[13]

Larvicidal activity

Culture of larvae

The *Artemia salina* seeds were obtained from Marina labs, India. The seeds were incubated in marine water for 48 hours for hatching in a small water tank. Aeration was provided with an aerator pump. Required light was provided with Philips 40 Watts lamp for 12 hours cycle . After 48 hours, the larvae were removed and used for the experiments. The hatched larvae were used in the nauplii stage.[26]

Bioassay

Larvae of *Artemia salina* were taken in different test tubes containing the extracts of *Symplocos cochinchinensis* , leaf and bark powder at different concentrations. Then five concentrations (1,2,3,4.5 ml) of each extract were added to 10 ml of sea water and 20 larvae were added to each of the test tubes. After 24 and 48 hours the viability of the larvae was recorded. The test tubes were maintained in triplicates. At the end of the experimental period, the numbers of mobile and dead larvae in each test tube were checked using a hand lens. Nauplii were considered dead when they were immobile and stayed at the bottom of the test tube.[26]

RESULT

The present study was done to find out the phytochemicals, anti oxidant activity, free radical scavenging activity and the toxicity assay of the methanolic and ethanolic extracts of the leaf and bark powder of *Symplocos cochinchinensis.* It was found that the ethanolic extracts of the leaf and bark showed the presence of phytochemicals like flavonoids, tannins and saponins. (Table 1) Both the extracts had significant larvicidal activity. The % mortality of the ethanolic extract of the leaf powder at the concentration of 2 mg/ml was 100 in the 24th hour whereas the methanolic leaf extract showed 100% mortality at 24th hour at the concentration of 3 mg/ml. Similarly methanolic and ethanolic extracts of the bark showed 100% mortality at 3 mg/ml in the 24th hour.(Table 2, Figure 3) At the 48th hour ethanolic extracts of both the leaf and bark showed 100% mortality at 2 mg/ml, whereas the methanolic extracts showed the same at 3 mg/ml.(Table 3 , Figure 4) The ethanol water extracts of the leaf and the bark showed maximum larvicidal activity at 24th and 48th hour.The total phenolic, total flavonoid, total antioxidant content were estimated. The total phenolic content was expressed as milligrams of tannic acid equivalents per gram of dry weight(mg TAE/gDW)of the plant using a standard plot of tannic acid. The ethanol water leaf extract had the maximum phenolic content of 13.81$\pm$0.21. (Table 4, Figure 5,6,7,8)The total flavonoid content is expressed as milligrams of Quercetin equivalent per gram dry weight (mg QE/gDW) of the plant material. The methanol extract of leaf had the maximum flavonoid content with 6.34 $\pm$0.21.(Table 5, Figure 5,6,7,8)The total antioxidants in the plant were expressed as mg TAE/g DW of the plant material. The methanol extract of leaf had the maximum antioxidant content with 16.34 $\pm$0.21.(Table 6,

Figure5,6,7,8) The methanol extracts of leaf and bark showed maximum antioxidant activity. Presence of phenols and polyphenols show remarkable activity against free radicals. In the present study the methanol and ethanol extracts of the leaf and bark of *S.cochinchinensis* was found to have free radical scavenging activity as shown by the DPPH assay.The free radical scavenging activity of the plant extracts was assessed by FTC,ABTS,TBA AND FRAP assays. During oxidation processes peroxides are converted into molecules of lower weight, which are measured by FTC and TBA methods.the methanolic extract of the leaf of *S.cochinchinensis* has shown potent ability against the primary and secondary products of lipid peroxidaton with 84.54% inhibition in FTC method and 88.89% in the TBA method. In FRAP assay the complex containing ferric ions is converted into ferrous ions due to the action of reducing agents-antioxidants thereby forming a chromogenic complex. In the present study it was found that the methanolic extract of the bark of *S.cochinchinensis* reveals the ability to reduce ferric ions (73.74%). Stable radicals of ABTS are generated when ABTS is missed with potassium persulphate and incubated in the dark. It is seen that the ethanolic water extract of *S. cochinchinensis* scavenged the radicals to a higher extent (55.71%) when compared with other extracts. The antioxidant assays performed represent the potential inhibition of the products of lipid peroxidation as well as the ABTS radicals by the ethanol and methanol extracts of the leaf and bark of S.cochinchinensis. The result recorded for FTC,FRAP,TBA and ABTS is presented in Table 7,8,9,10,11, Figure 9,10.

Table 1
Phytochemical analysis

	Leaf		Bark	
Solvent	**Methanol**	**Ethanol-Water**	**Methanol**	**Ethanol-Water**
Phytochemical				
Flavonoids	-	+	-	+
Tannins	+	+	+	+
Saponins	-	+	-	+
Terpenoids	-	-	-	-
Cardiac glycosides	-	-	-	-
Steroids	-	-	-	-
Phlobatannins	-	-	-	-

+ indicates presence
- Indicates absence

Table 2
Larvicidal activity 24th hour

Plant part	Solvent	% mortality Concentration (mg/ml)					*LD_{95} (mg/ml)
		1	2	3	4	5	
Leaf	Methanol	60	65	100	100	100	2.001
	Ethanol-Water	70	100	100	100	100	1.001
Bark	Methanol	75	90	100	100	100	2.02
	Ethanol-Water	70	85	100	100	100	2.007

Values are representatives of mean(n=3)

Table 3
Larvicidal activity 48th hour

Plant part	Solvent	% mortality Concentration (mg/ml)					LD_{95} (mg/ml)
		1	2	3	4	5	
Leaf	Methanol	70	80	100	100	100	2.003
	Ethanol-Water	80	100	100	100	100	1.003
Bark	Methanol	80	95	100	100	100	2
	Ethanol-Water	90	100	100	100	100	1.02

**Values are representatives of mean (n=3)*

Table 4
Total phenolic content

Plant part	Solvent	TPC (mg TAE/g)
Leaf	Methanol	13.67±0.29
Leaf	Ethanol-Water	13.81±0.21
Bark	Methanol	11.67±0.25
Bark	Ethanol-Water	13.43±0.24

Table 5
Total flavonoid content

Plant part	Solvent	TFC (µg QE/g)
Leaf	Methanol	6.34±0.21
Leaf	Ethanol-Water	1.23±0.25
Bark	Methanol	3.63±0.15
Bark	Ethanol-Water	1.93±0.2

Table 6
Total antioxidant content

Plant part	Solvent	TAC (mg TAE/g)
Leaf	Methanol	6.34±0.21
Leaf	Ethanol-Water	1.23±0.25
Bark	Methanol	3.63±0.15
Bark	Ethanol-Water	1.93±0.2

Table 7
DPPH free radical scavenging assay

Plant part	Solvent	10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	*EC_{50}
Leaf	Ethanol-water	34.34	56.57	62.63	83.84	97.98	29.96267
Bark	Methanol	7.07	33.33	49.5	63.64	83.84	31.41443
Bark	Ethanol-water	7.07	27.27	33.33	56.57	84.85	30.02581
Leaf	Methanol	20.05	29.87	37.33	47.78	59.67	4.037885

* ***Values are representatives of mean (n=3)***

Table 8
Ferric thiocyanate assay

Plant part	Solvent	% Inhibition
Leaf	Methanol	84.54+0.3
Leaf	Ethanol-Water	18.56+0.24
Bark	Methanol	22.68+0.17
Bark	Ethanol-Water	46.39+0.3

* ***Values are representatives of mean + SD (n=3)***

Table 9
Thiobarbituric acid assay

Plant part	Solvent	% Inhibition
Leaf	Methanol	88.89+0.25
Leaf	Ethanol-Water	62.63+0.21
Bark	Methanol	82.83+0.3
Bark	Ethanol-Water	64.65+0.5

Values are representatives of mean + SD (n=3)

Table 10
Ferric reducing antioxidant power

Plant part	Solvent	% Inhibition
Leaf	Methanol	17.17+0.2
Leaf	Ethanol-Water	7.07+0.13
Bark	Methanol	73.74+0.2
Bark	Ethanol water	64.65+0.09

* ***Values are representatives of mean + SD (n=3)***

Table 11
ABTS free radical scavenging assay

Plant part	Solvent	% Inhibition
Leaf	Methanol	42.86+ 0.05
Leaf	Ethanol-Water	55.71+ 0.21
Bark	Methanol	57.14+0.5
Bark	Ethanol-Water	38.57+0.23

* ***Values are representatives of mean + SD (n=3)***

Figure 1

Figure 2

Symplocos cochinchinensis leaf

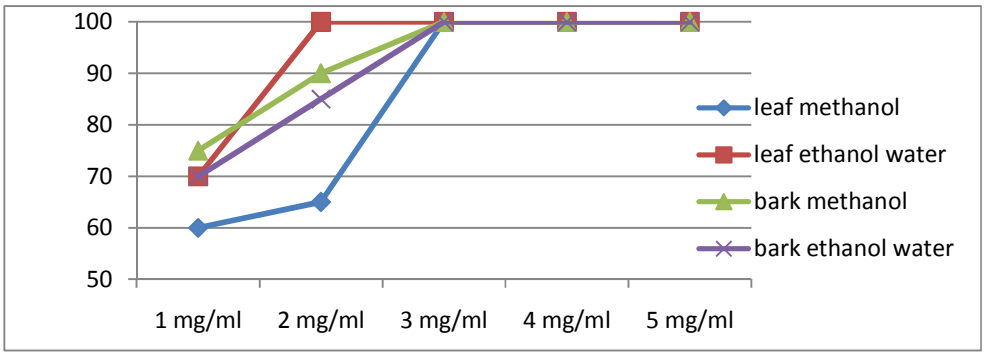


Figure 3

Larvicidal activity (24th hour) at the different concentrations by the ethanol and methanol extracts of the leaf and bark powder of Symplocos cochinchinensis.

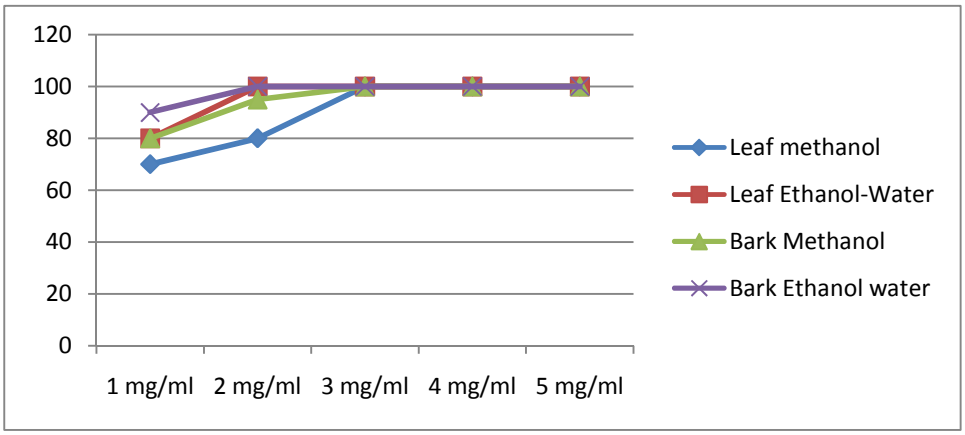


Figure 4

Larvicidal activity (48th hour) at the different concentrations by the ethanol and methanol extracts of the leaf and bark powder of Symplocos cochinchinensis.

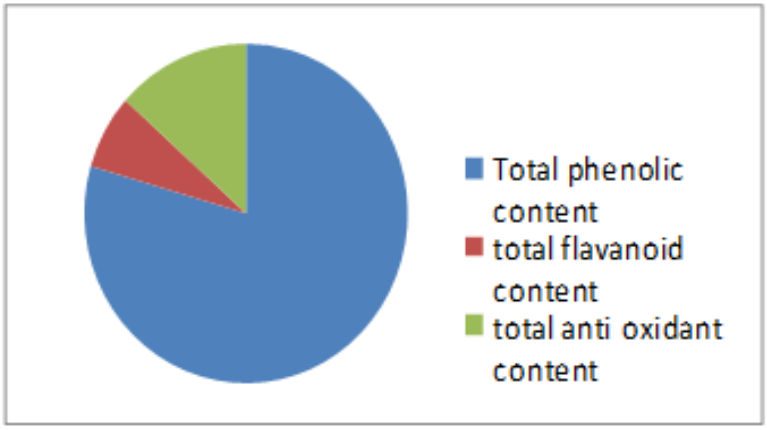


Figure 5
Total Phenolic, Flavonoid and Antioxidant content in the Ethanol extracts of the leaf powder

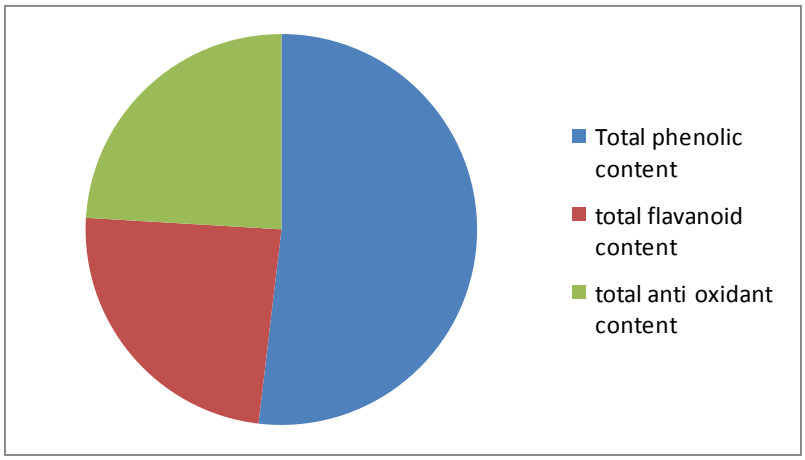


Figure 6
Total Phenolic, Flavonoid and Antioxidant content in the Methanol extracts of the leaf powder

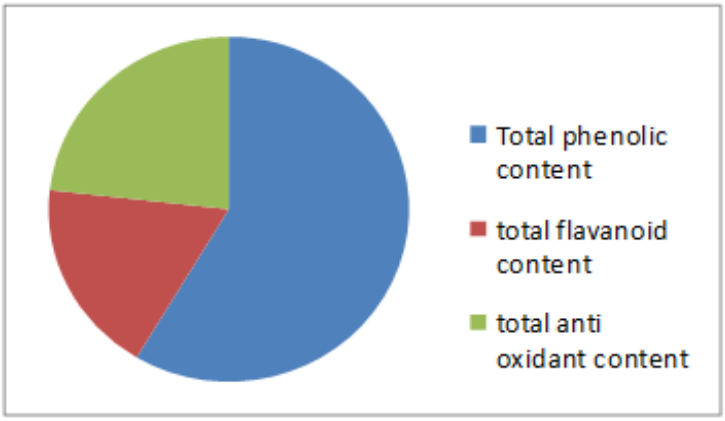


Figure 7
Total Phenolic, Flavonoid and Antioxidant content in the Methanol extracts of the Bark powder

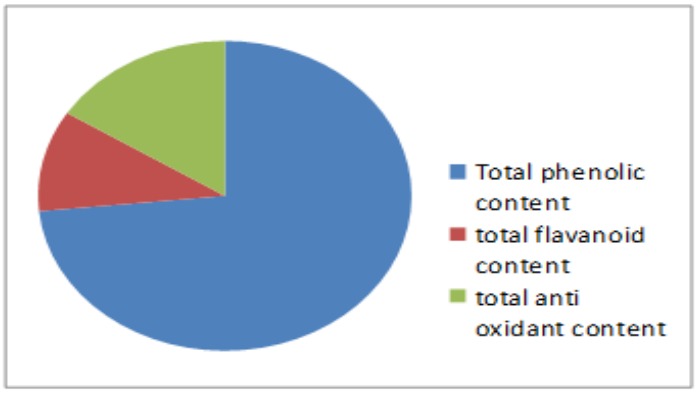
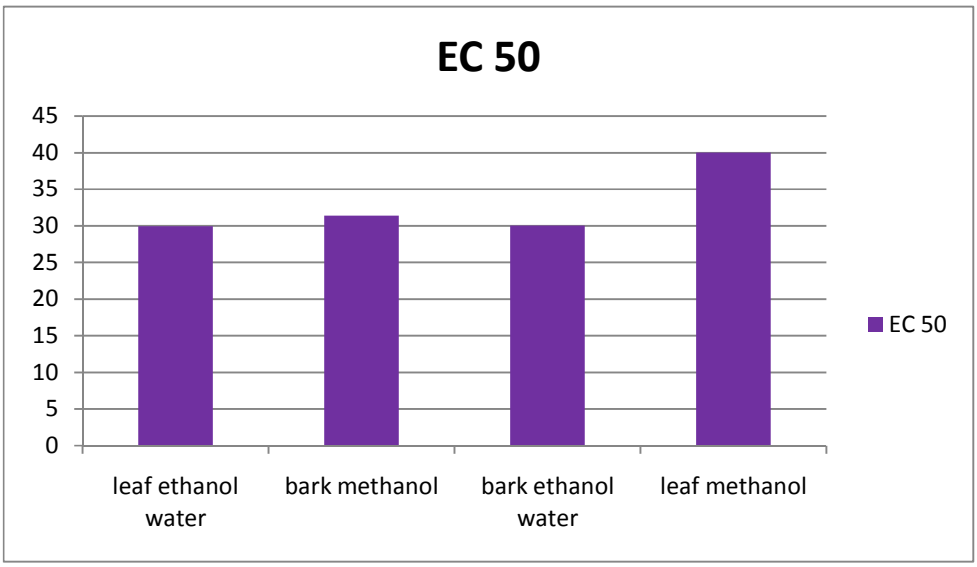


Figure 8
Total Phenolic, Flavonoid and Antioxidant content in the Ethanol extracts of the bark powder



Values are representatives of mean ± SD (n=3)

Figure 9
DPPH free radical scavenging activity of the ethanol and methanol extracts of the leaf and bark of Symplocos cochinchinensis

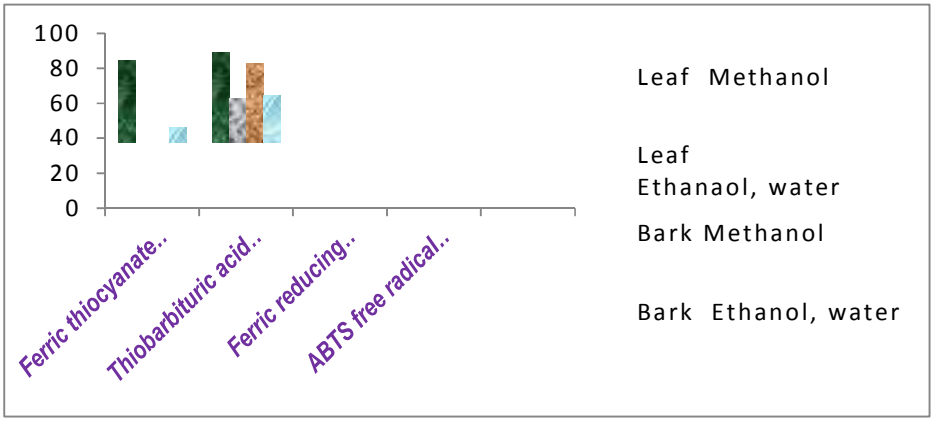


Figure 10
Antioxidant assays of the Methanolic and Ethanolic Extracts of the leaf and Bark powder.

The results are the average of the triplicates.

DISCUSSION

Medicinal plants have been traditionally used in the treatment of various diseases. Asian traditional medicinal systems such as Chinese medicine (TCM), Korean-Chinese medicine, Japanese-Chinese medicine (kampo), Ayurveda from India and Jamu from Indonesia are well known systems to cure various ailments.[1]In India, various plant extracts are used to cure tumors. Recently, different plant extracts are tested for their anti inflammatory , anti cytotoxic and anti tumor activities. Many plant species are still unexplored, the reason being the lack of access to these plants.[4]One such genus is *Symplocos*. The present study was aimed to study t the anticytotoxic activity of *S.Cochinchinensis*-an evergreen tree found at the higher altitudes of Western Ghats. Various studies prove that methanol helps in the extraction of different phytoconstituents and WHO has recommended extraction with ethanol: Water in the ratio 1:2 to be safe for human consumption. So, in the present study, the methanol extract and the ethanol water extracts of the bark and leaves of *S.Cochinchineasis* have been used. Flavonoids seem to have an important role in human food since they have medicinal properties. They are found only in plants and they have antioxidant, anticancerous, cardiotonic, capillary fragility and antithrombic properties. They are also reported to lower cholesterol, protect the liver and the stomach.flavonoids are reported to be anti-inflammatory, antimicrobial and analgesic.[27]The phytoconstituents in the extracts were assayed using the standard protocol and the results were found to be consistent with the work done by Sofia Banu *etal*(2013)The Brine shrimp lethality assay shows the cytotoxic activity of the plant extracts and this is supposed to be the pioneering attempt with this plant species. The cytotoxic activity was found to be effective and thus promise this plant species to be an effective anti cancer agent. The bioefficacy of a plant depends on its phytoconstituents. In the investigation of the biological activity of the plant extracts and the natural products, the assay on *Artemia salina,* brine shrimp larvae is a valuable tool for establishing the cytotoxicity and toxicity parameters.[26] Thus the lethality towards brine shrimp larvae is recommended as an effective prescreen for the existing invitro cytotoxicity and antitumor assays.[27]The standard antioxidant assays were done with the leaf and bark extracts. The methanol extracts of the leaf and bark showed maximum inhibition. This is so supportive in accordance to the report given by Sofia Banu *etal(2013)*. Similarly the different extracts of the leaf and bark of *Symplocos cochinchinensis* were found to have free radical scavenging activity which is the major indicator for the presence of antioxidant compounds in the extracts.

CONCLUSION

Many new plant species are being tested for their anticancer properties. Many factors are vital to prove their potential anti cancer activity. Any plant part which has shown the presence of certain bioactive compounds is subjected to further investigations. The ethanolic and methanolic extracts show significant lethality to brine shrimp larvae, at a very low concentration , making them potential candidates for further cytotoxic assays. In the present study the preliminary analysis for the antioxidant activity and the phytochemical constituents of the methanolic and ethanolic extracts of the leaf and bark of *S.cochinchinensis* was done. Based on the results, it can be concluded that these plant parts can be subjected to further investigations to find out their potential as effective drugs.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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