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Natural dye from *Careya arborea* Bark: Characterization and application in textile dyeing

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Abstract: *Natures indeed serves as a rich storehouse of natural colorants for various industrial applications. Natural colourants obtained from renewable sources are eco-friendly, risk free, nontoxic, have a curative effect and synchronized with nature. Disposal of natural dyes does not pose any problems to environment, easily biodegradable and highly compatible with the environment hence, demonstrated to have a vast applications in the textile industry to produce antimicrobial, deodorant and insect-repellant surfaces. Careya arborea is one such tree species in addition to its dye yielding ability, its different parts reported to have significant biological activities essential for textile dyeing. Hence, the present study is attempted to extract natural dye from the bark of C. arborea using various organic solvents such as methanol, acetone and petroleum ether. Methanol gave higher dye yield of 38.6 ±0.02% followed by acetone with 11.3±0.001% and petroleum ether yielded 3.88±0.05%. The methanol extract elicited flavonoids, tannins, quinines, sterol, phenol, anthocyanin, steroids and terpenoids of which phenol was in high quantity (10.471±0.010 mg/g). GC MS-MS analysis revealed the presence of 1,2-Ethanediol (30.71%),Hydroxylamine (29.77%) and Octahydro-1,1-dimethyl-6,6-ethylenedioxy-4a (hydroxymethyl) naphthalene (10.87%) with anti-oxidant, anti-microbial and anti-cancerous activities. The antioxidant and antimicrobial activities of methanol extract of C. arborea bark with significant free radical scavenging activity (81.93±0.001%) and 12MM to 16 MM zone of inhibition against tested pathogens respectively pave the way for using the bark extract of C. arborea in medical, textile and cosmetic products like hair dye and lipstick. C. arborea with chitharathai as mordant produced significant brownish orange colour with substantial colour fastness on fabrics and was confirmed with The South India Textile Research Association (SITRA), Coimbatore. Hence, the dye extracted from the bark of C. arborea can be considered to use in textile and cosmetic industries.*

Key Words: *Careya arborea, bark, secondary metabolites, extraction, natural colorant.*

1. INTRODUCTION:

Plants are major contributors to human welfare since the dawn of civilization and they play an important role to humankind not only economically, environmentally and industrially but also spiritually, historically and aesthetically as they sustain human life through direct and indirect gains by providing a wide range of products for survival and prosperity. In addition, plants are store house of natural colorants for various industrial applications[1]. Now a days, interests on natural colorant is increasing on the global level due to their environment friendly nature. Consumer awareness about the demerits of synthetic colorant, the health benefits of natural colorant and due to the worldwide tendency towards the consumption of natural products lead to significant increase in interest towards natural colorant and the research in the field. As a result, eco-friendly non-toxic, unsophisticated, naturally occurring bio-colorants have gained re-emergence with vast applicability and are an integral part of human life since time immemorial. Natural colorant are extracted from renewable sources such as plant materials, insects, algae, etc. The ability of natural dyes to color textiles has been known and to provide fundamental benefits in respect of the natural environment. The use of

plant dyes in the textile industry is challenging for multiple reasons, primarily with regard to questions of procurement, logistics and product quality. This type of natural dyes are being biodegradable and highly compatible with the environment when compare to artificial dyes. They have the promising biomolecules that are vital for the functional finishing of textile materials. Careya arborea is one such tree species in addition to its dye yielding ability the entire plant and its different parts have a long history of being used for enormous medicinal uses in Ayurveda and Chinese medicine.

Careya arborea Roxb. is a medium sized deciduous tree belonging to the family Lecythidaceae, an ethnomedicinal plant known as kumbhi in Ayurveda. It can be identified by its thick dark grey bark which observed to have dyeing character and contains various kinds of secondary metabolites like phenol, flavonoid, steroid and anthocyanin. Presence of these compounds accounts for various medicinal properties like hepatoprotective, antioxidant activity [4], antimicrobial [2], anti-inflammatory, analgesic activity [5] and antitumor [3]. Rathod et al., [6] reported that C. arborea leaves can be a potential source to probable anti-tumor and antibacterial agent. Kumar et al. (2006)[2] reported that methanol extract of C. arborea stem barks evaluated in various in-vitro systems showed broad spectrum antifungal activity. C. arborea reported to have strong antioxidant activities due to the presence of high level of phytochemicals such as phenolics and flavonoids which confirm its traditional claims as a source of potential antioxidant [7]. C. arborea with promising biomolecules with significant biological properties such as antioxidant and antimicrobial activities can be used as natural colourant for textile dyeing for application in medicinal field. Hence, the present study aimed to extract natural dye present in the bark of Careya arborea having medicinal properties such as antimicrobial and antioxidant activities for use as natural dye for textile industries.

2. Materials and Methods :

2.1. Sample Collection and Extraction

The barks samples of C. arborea were collected from tree situated between 10°41'23.3"N latitude and 79°10'12.3"E longitude at Sadivayal, Coimbatore falling under Cauvery Delta Agro climatic Zone of Tamilnadu. The bark sample collected was labeled properly and brought to the laboratory, cleaned in tap water and shade dried at room temperature. The processed bark was ground into fine powder and stored for further analysis. The powdered C. arborea bark samples (20 g each) were extracted using both polar and non solvents viz., methanol, acetone and petroleum each by both hot extraction in Soxhelt apparatus for 6-8 hours and cold extraction by soaking the bark powder in organic solvents for 24 hours The extracts were filtered and concentrated in rotary evaporator and stored at 4° C until further analysis.

2.2. Qualitative analysis of *C. arborea* bark extracts

Preliminary qualitative phytochemical screening of C. arborea bark was tested for the presence of major chemical constituents using the standard procedures described by [8].

2.3. Quantitative analysis of bark of *C. arborea*

Quantitative analysis of C. arborea bark extract was carried out by performing standard estimation procedures for phenols [9], flavonoids [10], steroids [11], tannins [12] and anthocyanin [13].

2.4. Thin Layer Chromatographic profiling of *C. arborea* bark extract

Thin layer chromatography analysis for phenol and flavonoids were performed by spotting concentrated, purified phytochemicals dissolved in diethyl ether on preparative silica gel-G plates coated with UV254 & UV356 binder (20 X 5cm, 250 µm with inorganic binder and UV 254) with 5 µl capillary pipettes and followed by running in glass chamber with liquid phase. Chromatographic spots were visualized using ultraviolet lamps emitting at 254 and 365 nm. The separated individual fractions eluted from TLC plates were purified in ethanol, filtered and stored at 4ºC.

2.5. GC/MS/MS analysis of *C. arborea* bark

TLC eluted phenolic fractions were filtered and the filtrate was evaporated to dryness. Added 3 ml of HPLC grade methanol and vortex for a minute, filtered and evaporated to dryness. The process was repeated fro three times. Finally the extract was purified by filtering through solid phase extraction cartridge. Injected 1µl of sample into the instrument for analysis. Interpretation on mass spectrum GC-MS/MS were conducted using the database of National Institute Standard and Technology (NIST) library having more than 62000 patterns. The spectrums of unknown compounds were compared with the spectrum of known compounds stored in the NIST library. The name, molecular weight and structure of the components of the test samples were ascertained.

2.6. Antioxidant activity of C. arborea bark using DPPH assay

The DPPH (1, 1-Dipheny- 2-Picrylhydrazyl) radical is used for the analysis of scavenging activities of many natural compounds. Ascorbic acid at various concentrations (20 to 100mg/ml) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity [15]. The capability to scavenge the DPPH radical was calculated using the following equation.

2.7. Antimicrobial activity of C arborea bark extracts using agar well diffusion method

Autoclaved molten Muller Hinton (MH) agar medium were poured in sterilized petri plates and allowed to solidify. Test organisms like *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilus* and *Klebsiella pneumoniae* were inoculated in sterilized nutrient broth and incubated at 37° C for 24 hrs. The petri plates containing MH medium were swabbed with 24hr microbial culture using sterile cotton swabs. Wells of 5mm diameter were made on Muller Hinton Agar plates using cork borer. Using a micro pipette, 20 µl of each plant extract (separately for methanol and acetone extract) was added on to each well on all plates. Streptomycin solution 1mg/ml used as the positive control and incubated at 37ºC for 24 hrs. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well [14].

2.8. Industrial applications of C. arborea bark extract

Textile application

Dyeing is the main process where the white natural material was decorated with attractive colors. 15 x 15cm sized dry white cotton cloth was treated with 10% NaOH at 630C for 30 minutes in a boiling water bath for the starch removal propose. Experiments were performed with and without mordant. In without mordant method, the processed cloth was treated with 5% Na2SO4 at 105o C for one hour in boiling water bath, washed and dried. Chitharathai (Alpinia officinarum) and Masikai (Quercus infectoria) and Alum as synthetic mordant were used for dyeing the fabrics. Mordanting was carried out in three stages, pre-mordanting, simultaneous mordanting and post-mordanting.

Pre-mordanting

In pre-mordanting, the scored fabrics were first treated with mordant and then dyed using extracts. The fabrics were treated with each of the mordants mentioned above in the ratio of 1:10 ml for 30 minutes at 50˚C. Then the mordanted fabric was used for dyeing.

Simultaneous mordanting

In this method, the fabrics were immersed in equal mixture of the mordant and the dye extract for 30 min at 50˚C followed by washing and drying of the dyed fabrics.

Post-mordanting

In case of post-mordanting, the dyed fabric was treated with mordants at 50˚C for 30 min in the ratio1:10.

3. Result and Discussion :

3.1. Extraction yield percentage

Plant derived compounds have peaked the interest of Nutrition Researchers in recent years. Increasing demand for natural supplements consumers stimulate demand for natural ingredients. The basic parameters influencing the quality of an extract are the plant parts used as starting material, the solvent used for extraction and extraction procedure. The C. arborea bark showed higher yield in hot extraction than cold extraction (Table 1). Gahlot [16] reported that the extract yield was high in hot extraction of jamun leaf (22.50 %) than cold extraction which yield only 9.25% and he observed similar results in curry leaves, litchi leaves and by product of catechu. The C. arborea bark extracted with methanol showed higher yield (38.6 ±0.02%) than acetone (11.3±0.001 %) and petroleum ether (3.88 ±0.05%) (Table 1). High yield of extract was obtained by using methanol as solvent for extraction. In case of extraction of Limnophila aromatic the extraction yield was found to be high in methanol (26.06%) than that of ethanol (17.03%) and pure acetone (12.33%) which showed that the extraction yield increases with increasing polarity of the solvent used in extraction [17].

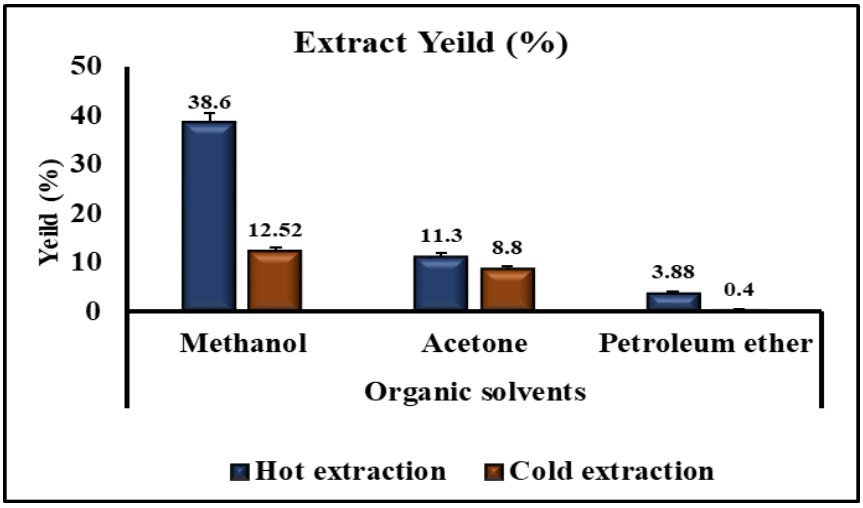


Fig 1: Extract yield of *C. arborea* bark extracted with organic solvents

3.2. Qualitative and quantitative analysis of secondary metabolites of *C. arborea* bark

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in a plant extract. The methanol extract of the C. arborea bark showed the presence of flavonoids, tannins, quinines, sterol, phenol, anthocyanin, steroids and terpenoids. In acetone extract flavonoid, tannin, sterol, phenol, anthocyanin, carbohydrate, steroids and terpenoids were present. In petroleum ether extract only flavonoids and terpenoids were eluted (Table 2). A variety of plant ingredients with diverse structures are capable of promoting health benefits. These secondary metabolites are widely used in human therapy, veterinary, agriculture, scientific research and in countless other area [18].

Table 1. Qualitative analysis of C. arborea bark extracts

Metabolites	Methanol	Acetone	Pet. ether
Alkaloids	-	-	-
Flavonoids	+	+	+
Tannins	+	+	-
Quinone	-	-	-
Sterol	+	+	-
Phenol	+	+	-
Anthocyanin	+	+	-
Protein	-	-	-
Carbohydrate	-	+	-
Steroids	+	+	-
Terpenoids	+	+	+
Glycosides	-	-	-

Note : + present, - Absent

Quantitative analysis of methanol and acetone extracts of C. arborea bark for phenol, steroid, tannin, anthocyanin and flavonoid revealed that phenol was quantified more in both the extracts (10.471±0.010 and 9.01 ±0.02mg/g) than other phytochemicals (Table 4). The C. arborea bark is reported to contain terpenes, sterols, tannins and saponins [19-20]. Antioxidant, antibacterial, antimicrobial, hepatoprotective, anticoagulant, analgesic, antidiarrheal, and other significant properties of C. arborea is due to the presence of phytochemical compounds [21].

Table 2. Quantitative analysis of C.arborea bark methanol and acetone extracts

Sample Extract	Phenols (mg/g)	Tannin (mg/g)	Flavonoid (mg/g)	Anthocyanin (mg/g)	Steroid (mg/g)
Acetone	9.01±0.02	9.22±0.002	6.55±0.03	1.25±0.02	7.40±0.003
Methanol	10.47±0.010	10.25±0.015	6.48±0.001	2.83±0.05	6.72±0.001

3.3. Thin Layer Chromatography (TLC) analysis of bark of *C. arborea*

TLC is used to analyze and characterize numerous natural compounds belonging to various chemical classes found in plant material i.e. crude plant extracts versus reference substances. In the present study separation of active components of bark of C. arborea using TLC showed the presence of phenols and flavonoids which would be responsible for the various biological properties. C. arborea subjected to extensive phytochemical research revealed the presence of a variety of phytoconstituents, particularly flavonoids [22]. Wadker [23] reported the presence of flavonoids and alkaloids in the bark of C. arborea.

3.4. GC-MS-MS analysis of bark of C. arborea

Recently there has been increasing interest in plants and plant derived bioactive compounds typically occur in small amounts and have more subtle effects as medicinal agents in various health care applications. GC MS-MS analysis was carried out for the identification of the phytochemicals present in the methanol extract of bark of C. arborea revealed the presence of bioactive compounds viz., 1,2-Ethanediol (30.71%), Hydroxylamine (29.77%) and Octahydro-1,1-dimethyl-6,6-ethylenedioxy-4a (hydroxymethyl) naphthalene (10.87%) with anti-oxidant, anti-microbial and anti-cancerous activities (Table 4). Many important bioactive compounds reported in the bark of C. arborea found to have a wide array of biological activities including anti-oxidative, anti-diabetic, **antimicrobial, anticancer, anti-inflammatory** [24].

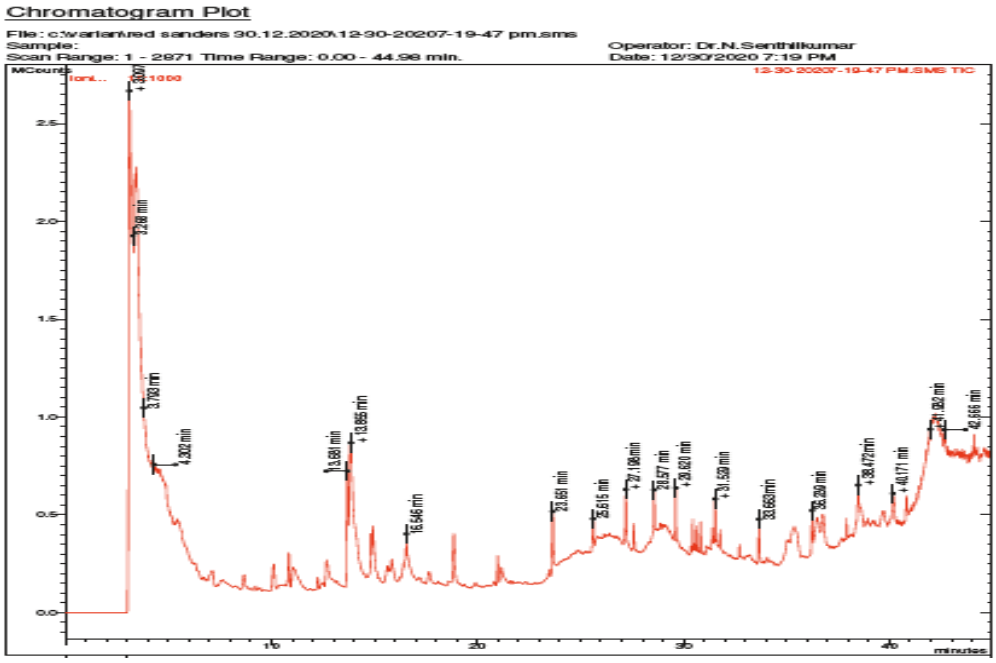
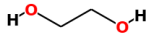
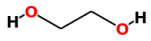
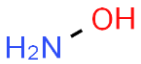
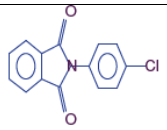
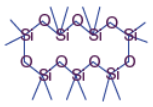
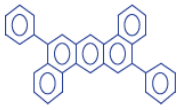
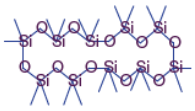
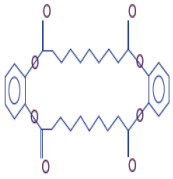


Fig 2. Chromatogram of methanol extract of C. arborea bark

Table 3. List of bioactive compounds identified in of *Careya arborea* bark through GC/MS/MS analysis

Compound name	RT	Percentage	Molecular weight	Molecular formula	Compound structure
1,2-Ethanediol (CAS)	3.097	30.714	62	$C_2H_6O_2$	
1,2-Ethanediol (CAS)	3.268	5.094	62	$C_2H_6O_2$	

Ethanol, 2,2,2-trifluoro-	3.372	10.874	100	$C_2H_3F_3O$	
Hydroxylamine	3.409	29.770	33	H_3NO	
H-Isoindole-1,3(2H)-dione, 2-(4-chlorophenyl)	13.855	5.365	257	$C_{14}H_8ClNO_2$	
Tetradecamethylcycloheptasiloxane	14.824	0.444	518	$C_{14}H_{42}O_7Si_7$	
Dibenz[a,h]anthracene, 5,12-diphenyl-	23.651	1.429	430	$C_{34}H_{22}$	
Eicosamethylcyclodecasiloxane	27.198	1.383	740	$C_{20}H_{60}O_{10}Si_{10}$	
8,9,10,11,12,13,14,24,25,26,27,28,29,30-Tetradecahydro-7H,23H-dibenzo [b,p][1,12,15,26] tetraoxy cyclooctacosin-6,15,22,31-tetraone	40.171	1.278	552	$C_{32}H_{40}O_8$	

3.5. Antioxidant activity of C. arborea bark using DPPH assay

Free radical scavenging activities of methanolic extract of C. arborea was assessed by using 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH), superoxide anion radical, nitric oxide radical and hydroxide radical scavenging assays. The antioxidant activity of the methanol extract of C. arborea (MECA) increased in a concentration-dependent manner proved that C. arborea can be a potential source of natural antimicrobial and antioxidant agents [2]. The radical scavenging activity of methanolic and acetone extracts was determined from the reduction in the optical absorbance at 517 nm due to scavenging of stable DPPH free radical. Positive DPPH test proposes that the examples are free extreme foragers. Many plant removes and their bioactive phytochemicals have shown free extremist rummaging properties [29-30]. yet there is a popularity to discover more data in regards to cancer prevention agent probability of numerous therapeutically significant plants. MECA had significant scavenging effects on the DPPH radical and the effects increased with increasing concentration in the range 200-1000 □g/mL (Fig 3). The methanol and acetone extract of bark of C. arborea showed high DPPH radical inhibition in 1000□g/ml and methanol extract showed high percentage (81.93±0.001%) of DPPH activity than acetone extract (79.62%±0.003) (Table 6). The methanol extract showed strong antioxidant activity against many oxidants in the in vitro antioxidant screening. The presence of flavonoids, phenols and polyphenolic compound in bark of C. arborea incited us to examine the free revolutionary rummaging action which demonstrated the bark and of C. arborea as a promising wellspring of normally happening cancer prevention agent for restorative and business employments. The bioactive mixtures would be liable for the different impacts played by C. arborea in the medicines of a few infections might be because of the individual or joined activity of the dynamic phytochemicals. By this result the antioxidant activity is due to the presence of phenolic and flavonoid content and also use to cure various diseases [31]. From the above studies we suggested that methanolic extract of C. arborea could be used as a source of natural antioxidant and broadly utilized for the therapy of some degenerative illnesses therapy. This could ultimately lead to the inclusion of this com-pound(s) in different antioxidant pharmaceutical formulation [32].

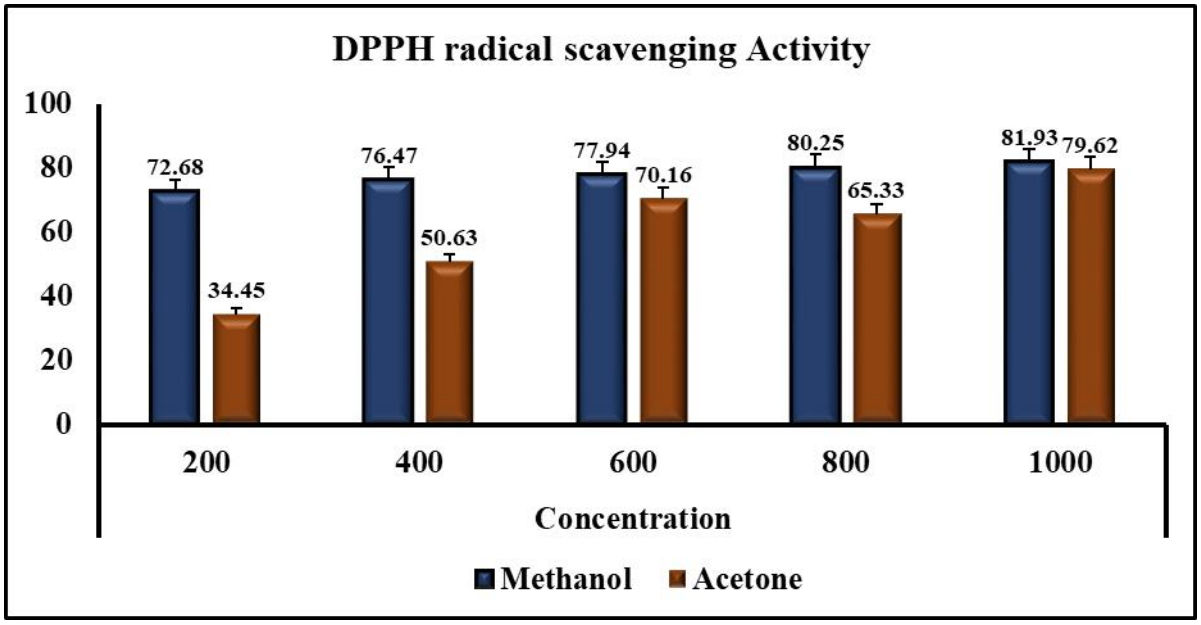


Fig 3: DPPH scavenging activity of bark of *Careya arborea*

3.6. Antimicrobial activity of C arborea bark extracts using agar well diffusion method

Antimicrobial activity of methanol extract of C. arborea was carried out using disc diffusion methods with Gram positive and Gram negative bacteria showed broad-spectrum antimicrobial activity against all tested microorganisms [2]. The methanol extract of C. arborea bark showed antibacterial study against the tested pathogens Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli and Bacillus subtilis along with Streptomycin at a pH 7 as positive control (Fig 2). After the incubation period of 24 hours, there should be a zone of inhibited growth around the reservoir, whose size is related to the antimicrobial capacity of the substance. The inhibition rate of Klebsiella pneumonia was16MM followed by Escherichia coli with 15MM, Staphylococcus aureus with 14MM, Bacillus subtilis and Pseudomonas aeruginosa showed 12MM inhibition (Table 5). The leaf extract of C. arborea exhibited significantly lower zones against S. aureus and E.coli [25] Methanol extract of C. arborea stem barks evaluated in various in-vitro systems showed significant antibacterial against all tested Gram positive and Gram negative bacteria [2]. The bioactive compounds such as phenols, flavonoids and tannins reported in the bark may be attributed to its antibacterial activity. Flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms that Inhibition of nucleic acid synthesis [26] and Phenolics and polyphenols present in the plants are known to be toxic to microorganisms which may carry Cell wall disruption, enzyme denaturation [27]. These phyto-constituents may be responsible for the antimicrobial activity of C. arborea.

Table 4. Antibacterial activity of methanol extract of bark of *C. arborea*

Samples	***P.areuginosa***	***S.aureus***	***B.subtilis***	***E.Coli***	***K.pneumonia***
Streptomycin (+)	19±0.001mm	23±0.002mm	31±0.001mm	20±0.003mm	22±0.002mm
DMSO (-)	No zone	No zone	No zone	No zone	No zone
Methanol extract	12±0.002mm	14±0.001mm	12±0.001mm	15±0.002mm	16±0.004mm

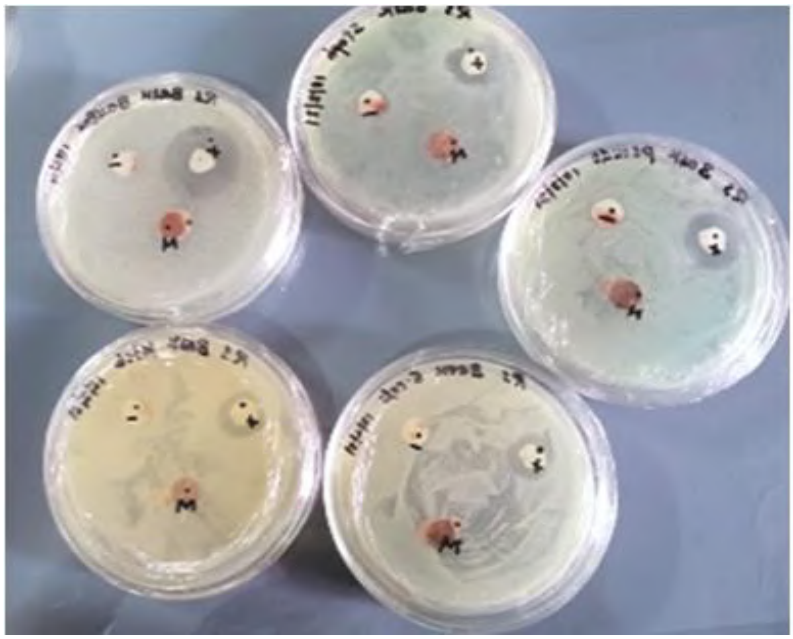


Fig 4. Antibacterial activity of methanol extract of bark of *C. arborea*

3.7. Fabrics/Textile dyeing using methanol extract of C. arborea bark

Methanol extract of C. arborea bark was used for dyeing cotton fabric without mordant and with mordant. C. arborea extract with chitharathai as mordant produced significant brownish orange colour when compared to without mordant. The dye extracted from C. arborea bark along with chitharathai mordant was highly efficient after three to four wash of dyed cotton fabric proved its colour fastness on fabrics. The result was reconfirmed with The South India Textile Research Association (SITRA), Coimbatore and the results confirmed that the dye from C. arborea with mordant chitharathai produced substantial colour fastness than without mordant. The colors of the plants are mostly due to the presence of aromatic oxygen containing pigments called flavonoids found in the epidermal region of plants. With the knowledge of the public towards eco-safety and health concerns, interest on natural dyes has been increased and hence eco-friendly products are gaining popularity. The advantage of these natural colorants revolves around the fact that they are usually agro renewable, biodegradable, and non-toxic [33].

Fig 5. Cotton fabric dyed with methanol extract of *Careya arborea*

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Address all correspondence to the Director

Test Report No : N2003936 ULR: TC694421300007337F Report Date : 04-03-2021

The Director *Ref : Dt.02.03.2021*

Colour Fastness to Washing : Test 3 *As per IS / ISO 105 - C10:2006 C(3)*	**N2003936-1** Sample Particulars : Cotton Fabric
Change in colour	4
Staining on	.
Viscose	4
Acrylic	4-5
Polyester	4-5
Nylon	4-5
Cotton	4-5
Tri Acetate	4-5

Grey Scale Rating :- Change in Colour: 5-No change, 4-Slightly changed, 3-Noticeably changed, 2 - Considerably changed, 1 - Much changed Staining: 5-No staining, 4-Slightly stained, 3-Noticeably stained, 2 - Considerably stained, 1 - Much stained.

- End of Report -

4. CONCLUSION

The individual or combined action of the bioactive phytochemicals presence in the bark of C. arborea may be responsible for the various medicinal properties. The presence of flavonoids, phenols and polyphenolic compound in bark of C. arborea prompted us to study the free radical scavenging and antimicrobial activities proved the bark of C. arborea as a promising source of natural dye with antioxidant and anti-microbial properties. C. arborea can be a potential sources of natural colourant for use in cosmetics and textile industry, since the bark extract was also evaluated for dyeing properties in textile along with natural mordant.

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