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Comparative antioxidant and antimicrobial studies of cold and hot bark hydromethanolic extract of *Couroupita guianensis Aubl*

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***Couroupita guianensis*, known by its common name as cannon ball tree, belongs to Lecythidaceae family, native of south and central America, India and Sri lanka. It's been in use in traditional medicine and worshipped as sacred tree in india. With the importance of tree in various medicinal aspects as per the reports the present investigation was carried to access the phytochemical components, free radical scavenging and antimicrobial activity of cold and hot hydromethanolic extracts from the barks of medicinally important *Couroupita guanensis*. Phytochemical screening revealed the presence of total antioxidants, flavonoids, phenols and phytosterols in both cold and hot extracts. Quantitative estimations of the phytochemicals revealed the presence of high contents of total antioxidant activity (*598.4* µg/ml), phenol content activity of (417.52 µg/ml) and phytosterols of (133.92 µg/ml) in cold hydromethanolic extracts compared to the hot hydromethanolic extract. The presence of high flavonoid content (417.52 µg/ml) was recorded in hot extract compared to the cold extract. Hot extract gave more scavenging activity with IC_{50} value (33.5 µg/ml). ABTS radical scavenging activity was found to be more in cold extract with IC_{50} values (24. µg/ml). Antimicrobial assay showed activity with *B. cereus* (13.00±0.00mm) and *S.aureus* (15.00±0.00mm) bacteria showed maximum zone of inhibition compared to the hot extract whereas *C. albicans* (13.00±O.00mm) showing maximum zone of inhibition compared to the cold extract and was not sensitive to any other fungal forms tested. This investigation pays way to consider *Couroupita guianensis*, with highly potential antioxidant and antimicrobial components, be used in pharmaceutical companies for the development of phytomedicine for the therapy and treatments.**

Key Words: *Couroupita guianensis*, Barck, extracts, phytochemical, free radical scavenging activity, antimicrobial assay.

Traditionally used medicinal plants have recently attracted the attention of the pharmaceutical and scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations (Taylor *et al.*, 2001). Many of the plant secondary metabolites are constitutive,

existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated in response to tissue damage or pathogen attack (Osbourne, 1996). *Couroupita guianensis* has lot of medicinal properties. *Couroupita guianensis Aubl* (Lecythidaceae) is commonly called as cannon ball tree. *Couroupita guianensis* possesses antibiotic, antibacterial, antifungal, anti inflammatory, antiseptic, antipyritic, hemostatic, analgesic qualities (Gauresh somani *et al*., 2012). It is used in treatment of cold, stomachache, hypertension, tumor, toothache, malaria, skin disorders and wounds (Sanz *et al*., 2009). Antioxidants acts as a defense mechanism that protects against oxidative damage and include compounds to remove or repair damaged molecules (Jaya chitra, 2012). Oxidative stress is a factor for many human diseases as either a cause or an effect. Plants are the source of medication for preventive, curative, protective or promotive purposes (Sidhu *et al*., 2007). Imbalance leads to damage of important biomolecules and organs with potential impact on the whole organism. Antioxidant can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress (Durackova, 2010). Natural antioxidants have been studied extensively for decades in order to find compounds protecting against a number of diseases related to oxidative stress and free radical-induced damage. To date, many plants have been claimed to pose beneficial health effects such as antioxidant properties (Kaur & Arora, 2009; Newman & Cragg, 2007). So the present investigation was started with an aim to evaluate the phytochemical components and free radical scavenging activity of cold and hot hydromethanolic extracts from the bark of medicinally important *Couroupita guanensis.*

Materials and Methods:

Preparation of the plant material and extraction: The bark of the plant was collected from natural habitat and rinsed with distilled water to remove the dust particles. The water was removed by blotting over a filter paper. Then the plant materials were shade dried and powdered. Ten gram of powdered plant material was weighed and taken in muslin cloth and made into packets. The packets were used for extraction by using hydro methanol respectively. The cold and hot extraction procedure was followed as per the previous studies (Nagananda *et al*., 2013)

Qualitative phytochemical screening:

The different qualitative chemical tests were performed for establishing phytochemical profile of hydro methanolic extracts obtained from cold and hot extractions. The tests for alkaloids, Saponins, Phytosterols, phenols, Tannins, glycosides, flavanoids were performed on both the extracts to detect various phytoconstituents present in them (Raaman, 2006, Nagananda *et al*., 2013).

Estimation of phytosterols: The extracts were dissolved in chloroform and were estimated for phytosterols by libermann-burchard method (Finar, 1986) with absorbance measured at 640 nm with cholesterol (1mg mL^{-1}) as the standard.

Estimation of total flavonoid : The extracts were dissolved in DMSO and were estimated for total flavonoid content by aluminium chloride method (Zhishen *et al*, 1999) with absorbance measured at 510 nm with quercetin (100µg mL^{-1}) as the standard.

Estimation of total phenols: The extracts were dissolved in 5mL of distilled water and were estimated for total phenols by Folin-Ciocalteau reagent method (Sadasivam and Manickam, 1997) with absorbance measured at 650 nm with catechol (50 µg mL-1) as the standard.

Total Antioxidant Capacity: The extracts (1mg mL^{-1}) were dissolved in DMSO and were checked for total antioxidant capacity by phosphomolybdenum method (Prieto *et al.*,1999) with absorbance measured at 695nm and ascorbic acid (1mg mL^{-1}) serves as standard.

DPPH (1,1-diphenyl-2-picrylhydrazyl) Free Radical Scavenging assay (Blois,1958): Standard ascorbic acid and extracts (1 mg mL^{-1}) at various concentrations (10-50 µg) were taken and the volume were adjusted to 100 µL with methanol. Five millilitre of a 0.1mM methanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27˚C. The absorbance of the sample was measured at 517 nm. Methanol serves as a blank and the experiment was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

% DPPH radical scavenging activity = [(control OD – Sample OD)/ Control OD)] *100.

ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolourization assay (Re et al., 1999): ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS (7mM) with 2.45mM Ammonium persulfate and the mixture was allowed to stand in dark at room temperature for 12-16h before use. Standard ascorbic acid and sample extracts (1mg mL^{-1}) at various concentrations (10-50 µg) were taken and the volume was adjusted to 500µL with methanol and 500µL of methanol serves as blank. 300 µL of ABTS solution was added; the final volume was made up 1 mL with ethanol and incubated in dark for 30min at room temperature. The absorbance was read at 745nm and the experiment was performed in triplicate. Radial cation decolourization activity was expressed as the inhibition percentage of cation by the sample and was calculated using the formula:

% ABTS radical scavenging activity = [(control OD – Sample OD)/ Control OD)] *100.

IC_{50} value: IC_{50} value (concentration of sample required to scavenge 50% of free radical) were calculated from the regression equation, prepared from the concentration of the samples and percentage inhibition of free radical formation. Ascorbic acid was used as positive control and all tests were carried out in triplicate.

Statistical analysis: The experiments were set up in a completely randomized design. All values obtained from the mean replicates to the variance and presented as mean± standard error (SE). Analysis of variance was conducted by two ways ANOVA and the mean were compared by Tukey HSD test. All statistical analysis was performed at 1% significance level using IBM SPSS Statistics (version 20) by IBM.

Results

Qualitative phytochemical screening: The different qualitative chemical tests were performed for establishing phytochemical profile of extracts obtained from cold and hot hydromethanollic extraction. Phytochemical screening revealed the presence of flavonoids, phenols and phytosterols in both cold and hot hydromethanolic extracts Table: 1.

Table 1: Phytochemical screening of cold and hot bark hydromethanolic extract of *Couroupita guianensis*

Phytochemical Screening	Cold extract	Hot extract
Alkaloids		
Mayer's	+	+
Wagners	+	+
Hager's	+	+
Dragendroff's	+	+
Saponins		
Foam Test	-	-
Phytosterols		
Liebemann-Burchards	++	+
Phenols		
Ferric chloride	+	+
FC reagent	+	+
Flavonoids	+	+
Glycosides	-	-

Highly present : ++ , Present : + , Absent: -

Quantitative estimation of phytochemicals: The quantitative estimations of the phytochemicals, which were qualitative detected in the *Couropita guanensis* bark revealed the presence of high total antioxidant activity capacity *(598.4* µg/ml), phenol content (417.52 µg/ml) and content of phytosterols (133.92 µg/ml) in cold hydromethanolic extracts compard to the hot hydromethanolic extract. The presence of high flavonoid content (417.52 µg/ml) was recorded in hot hydromethanolic extract compared to the cold hydromethanolic extract (Table-2).

Table 2: Quantitative estimation of phytochemicals

Extracts	Phenols (µg/ml) X*± SE	Phytosterols (µg/ml) X*± SE	Flavonoid (µg/ml) X*± SE	Total Antioxidant (µg/ml) X*± SE
Cold Extract	417.52±0.030	133.92±0.11	57.12±0.056	598.4±0.021
Hot Extract	386.24±0.025	80.6±0.044	76.16±0.032	596.13±0.028

Note: * - Mean of 3 replications., SE- Standard Error

Free radical scavenging activity assay:

For DPPH free radical scavenging activity assay the highest percentage of scavenging activity (74.274%) and IC_{50} values (33.5 µg/ml) were found to be best in hot hydrometanollic extract. For ABTS radical scavenging assay the highest percentage of scavenging activity (68.28%) and IC_{50} values (24. µg/ml) was found to be best in cold hydrometanollic extract (Table-3) (Fig. 1,2).

Table 3: Percentage of scavenging activity and IC_{50} value of different extracts against different assays

Extracts	DPPH% of Scavenging	ABTS% of Scavenging	DPPH IC_{50} (µg mL^{-1})	ABTS IC_{50} (µg mL^{-1})
STD	46.44[c]	88.83[a]	32.08[a]	30.19[b]
Cold extract	63.68[b]	68.28[b]	79.38[c]	24.74[a]
Hot extract	74.27[a]	56.22[c]	33.50[b]	35.63[c]

Note: Mean of 15 replicate. Mean values with different superscripts ([a, b, c]) differ significantly at P<0.01 by Tukey (HSD) test

Table 4: *In vitro* anti bacterial and antifungal activities of 5 cold and hot hydromethanolic bark extracts on 4 bacteria and 5 fungi isolates showing diameters of the inhibitory zones (in mm)

Zone of inhibition (mm) for Anti bacterial activity X*± SE				
	E. coli	***B. cerreus***	***S. aureus***	***Psedomonas***
Cold extract	-	13±0.065mm	15±0.377mm	-
Hot extract	-	12±0.334mm	13±0.222mm	-
Standard	30±0.771mm	32±0.342mm	38±0.521mm	-
Control	-	-	-	-

Zone of inhibition (mm) for Antifungal activity X*± SE					
	A. niger	***A. flavors***	***C. albicans***	***Mucar***	***Pencillium***
Cold extract	-	-	11±0.623mm	-	-
Hot extract	-	-	13±0.543mm	-	-
Standard	-	-	32±0.432mm	-	-
Control	-	-	-	-	-

Note: * - Mean of 3 replications., SE- Standard Error

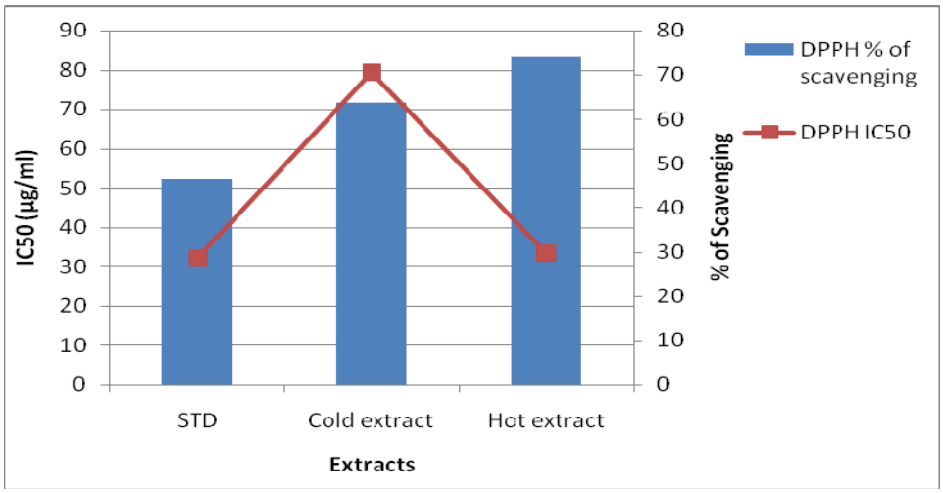

Fig. 1: DPPH scavenging activity of different extracts and its IC_{50} (µg mL^{-1})

Anti Microbial Activity :

Antibacterial activity studies showed that in cold Hydromethanolic extract the bacterial form *B.cerreus*(13.00±0.00mm) and *S.aureus*(15.00±0.00mm) was found to be more sensitive showing maximum zone of inhibition compared to the hot hydromethanolic extract (Table-4).

Antifungal activity studies showed that in hot hydromethanolic extract the fungal form *C.albicans*(13.00±0.00mm) was found to be more sensitive showing maximum zone of inhibition compared to the cold extract and was not sensitive to any fungal forms tested (Table- 4).

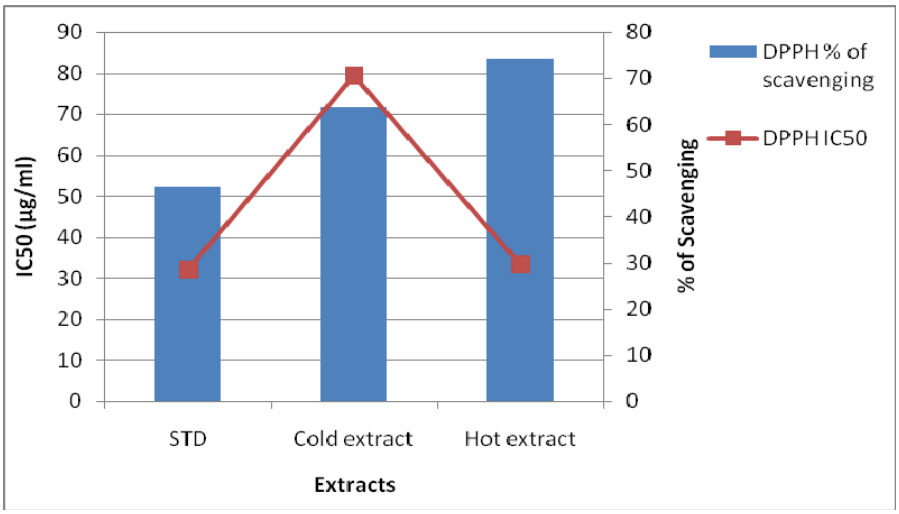

Fig. 2: ABTS scavenging activity of different extracts and its IC_{50} (µg mL^{-1})

Discussion

Previous studies on *Couroupita guianensis* shows its medicinal importance of different parts like fruit rind (Regina & Uma, 2012), roots (Juvekar, 2009), leaves (Sivakumar *et al*., 2012, Eluumalai *et al.,* 2012), flower (Pradhan, 2009) and not much work has been done on the stem and bark. So the present investigation was started with the aim to extract the phytochemicals from the bark through hot and cold extraction methods by using hydromethanollic solvent. The preliminary screening of secondary metabolites showed the presence of phenols and flavanoids which coincides with earlier studies (Regina & Uma, 2012). The presence of phytosterols were not reported earlier from any parts of the plant. The cold extraction was more effective for extracting phenols and phytosterols, whereas hot extraction was more effective for flavonoids.

Under certain environmental conditions and during normal cellular functions in the body the free radicals are produced (Nagananda *et al*., 2013). The produced free radicals creates the oxidative stress in the cells and leading to damage of the cells leading to various diseases. Antioxidants are the compounds which protects the cells against oxidative stress damage and also repairs the damaged cells (Jayachitra, 2012). In the present investigation, the antioxidant activity was determined with various assays. In DPPH assay, molecule involves in their hydrogen-donating ability has shown an evident high scavenging activity with the hot hydromethoonollic extract which might be due to the presence of some thermo stable flavonoids. ABTS molecules involves indirect generation of ABTS radical mono cation with no involvement of any intermediary radical has shown evident scavenging activity with the cold

extract which might be due the presence of thermoliable phenols and phytosterols with coincides with the findings of Nagananda *et al*. (2013).

Antimicrobial activity studies has been carried on with different parts of the plant and not much work has been done on the bark extracts. So in the present investigation the antibacterial and anti fungal activity against hot and cold bark hydromethonolic extract were studied. Antibacterial activity has shown that the cold extracts are showing the maximum zone of inhibition for *B. cereus* and *S. aureus*. The present activity on *S. aureus* coincides with the earlier finding with flower extracts (Ramalakshmi *et al*., 2013). Antifungal activity has shown that cold and hot extracts have shown the zone of inhibition for only *C. albicans* and was not active against any other fungi used. The result of antifungal activity coincides with the earlier findings with fruit extract (Al-Dhabi *et al*., 2012).

The present study indicates that *Couroupita guianensis bark extracts* contains large reservoir of phytochemicals, highly potential antioxidant and antimicrobial components that may be used for the development of phytomedicine for the therapy and treatments.

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