Studies on the phytochemistry and bioefficacy of industrial crops - *Coffea canephora* and *Gravillearobusta* from Kolli hills

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ABSTRACT

Industrial crops are used only for their potential plant parts in general, neglecting the other parts, which are either wasted or underutilized. If proper study is conducted on the neglected parts of industrially significant plants, it might result in further exploitation of the same in other allied industries. Hence, to know the potency of leaves, which are neglected parts, of the plants of *Coffea canephora* L. Linden and *Gravillea robusta* A. Cunn. Ex. R. Br. were studied for their phytochemistry, antibacterial, antioxidiant, larvicidal and pesticidal activities. The plant leaves were collected from Kolli Hills of Tamil Nadu, India. Acetone, Chloroform, Ethyl acetate and Methanolic extracts of the leaves of the plants were studied for their antibacterial efficacy through micro broth dilution method, antioxidant property using DPPH free radical scavenging assay, larvicidal activity using the larvae of *Artemia salina* and pesticidal potency using the storage pest, *Sitophilus oryzae*. The plant *Coffea canephora* has shown the presence of Tannin and Steroid and the plant *Gravillea robusta*, the presence of flavonoid and steroid. The plant *Coffea canephora* has showed better antibacterial activity when compared with *Gravillea robusta*. Similarly, aceton extract of *Coffea canephora* showed better antioxidiant ability. Mortality of 100% was recorded against *Artemia salina* and *Sitophilus oryzae* for all types of extracts of the plants studied. The study suggests the plant *Coffea canephora* as a potential member which can be exploited as an antibacterial agent. The study also suggests that the plants studied can be utilized in controlling the storage pest, *Sitophilus oryzae*.

Keywords: Kolli Hills; *Coffea canephora*; *Gravillea robusta*; Antibacterial; Antioxidant; Pesticidal

INTRODUCTION

Industrial crops are used only for their potential plant parts in general, neglecting the other parts, which are either wasted or underutilized. In our opinion, if proper study is conducted on the industrially neglected parts of the industrial plants, it may result in further exploitation of the same in other allied industries. Thus, in this study the leaves of two different industrial crops, *Coffea canephora* L. Linden (Rubiaceae) and *Gravillea robusta* A. Cunn. Ex. R. Br. (Proteaceae) grown in Kolli Hills in the state of Tamil Nadu, India, which are neglected industrially were selected. They were evaluated for their phytochemistry, antibacterial activity, antioxidiant ability, larvicidal and pesticidal potency.

Coffee is the most widely consumed beverage in the world and the most commercialized food product. The production of coffee has reached 8.1 million tons worldwide (ICO, 2011). The increase in coffee consumption is due to the information on its health benefits. The compounds of coffee exert antioxidiant and other beneficial biological properties. There are nearly 80 identified species of coffee (Clark, 2003). Among them, *Coffea arabica* accounts for nearly 70% of coffee market and the remaining by *Coffea canephora* (Robusta coffee) (ICO, 2011 and ABIC, 2011). Antioxidant activity of coffee was studied by Charurin et al., (2002) and Yen et al., (2005). Daglia et al., (1998 and 2007) has identified antibacterial compounds from roasted coffee. The antibacterial activity of coffee against Entrobacteria (Almeida, et al., 2006), Streptococcus mutans (Antonio et al., 2010) and oral bacteria (Antonio et al., 2011) have been reported. A detailed report on the chemical constituents of coffee was reported by Farah (2012). Most of the reports available are on coffee beans and the study on the leaves of the plant has been completely neglected. Thus, in this study, the leaves of the plant, *Coffea canephora* was studied for their bio-efficacy.

The silky oak (*Gravillea robusta*) belonging to the family Proteaceae is native of Australia and is widespread in many countries. The tree reaches a height of 12-25 m, sometimes 40 m. The plant is potentially used as tim-
ber and is widely used in making furniture. Antibacterial (Cock, 2007a), larvicidal (Cock, 2007b) and antioxidant activity (Samarth and Krishna, 2007) of the leaves and leishmanicidal activity of the bark (Takahashi, 2004) of the plant has already been reported. However, the previous studies mentioned were confined only with the methanolic extract and not with other types of solvents to study the bioactivity. Hence, in this study, other solvents like acetone, chloroform and ethyl acetate along with methanol were used to know the bioactivities of the plant leaves of *Gravilea robusta*.

**MATERIALS AND METHODS**

Plant Source

Kolli hills are a part of Eastern Ghats situated in district of Namakkal belonging to the State of Tamil Nadu, India. This is situated at the latitude of 10°12’ - 11°7’N and the longitude of 76° - 77°56’E. The altitude of the mountain ranges from 1000 to 1300 m and is spread over an area of 280 sq. km. Coffee, Jack, Pine apple, pepper and spices are the major cash crops grown in Kolli hills. In this study, the industrial crops, i.e. *Coffea canephora* L. Linden (Rubiaceae) and *Gravilea robusta* A. Cunn. Ex. R. Br. (Proteaceae) were studied for their bioefficacy.

Plant material preparation

Healthy, uninfected leaves were chosen and cleaned using running water. They were shade dried for a period of 4-5 days till they became crispy and were pulverized using electric blender and stored (Udayaprakash, 2013a and b).

Phytochemical analysis

The dried, pulverized plant materials (5 g) were extracted with double distilled water (100 mL) by boiling. The aqueous extracts were filtered using Whatman No.1 filter paper and the detection of phytochemicals like cardiac glycosides, flavonoids, phlobatannins, saponins, steroids, tannins and terpenoids was done according to standard procedures (Evans, 1996; Udayaprakash, 2011 and 2013c).

Solvent extracts

The leaf extracts of the plants were obtained using cold percolation method. Acetone, Chloroform, Ethyl acetate and Methanol were used as the solvents. To 20 g of each dried pulverized sample, 200 mL of individual solvent was added and stirred in a temperature controlled shaker at 30 ± 2°C for a period of 48h. The crude obtained were filtered, concentrated and reconstituted for evaluation of antibacterial, larvicidal, pesticidal and antioxidant properties.

Antibacterial assay

Minimum Inhibitory Concentration (MIC)

The antibacterial efficacy of industrial crops were studied against 4 bacterial strains, i.e. *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 443), Klebsiella pneumoniae (MTCC 1320) and *Vibrio paraahemolyticus* (MTCC 451) procured from Microbial Type Culture Collection and Gene Bank, Chandigarh, India.

The study was conducted using Micro broth dilution in 96 well titre plates. The plant extract of 100 µg/mL as the initial concentration was taken in the first well and this was serially diluted. The dilution resulted in 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.125 µg/mL, 1.6 µg/mL and finally 0.8 µg/mL in series (Udayaprakash, et al., 2014a). Streptomycin was used as the positive control. Each well with respective concentration of plant extract was inoculated with 0.01 mL of 24 hours bacterial cell suspension. This was incubated at 37°C for 24 hours. The presence of cloudiness or turbidity of the broth indicates positive growth. The concentration which inhibits the bacterial growth is considered as the Minimum Inhibitory Concentration (MIC).

DPPH free radical scavenging assay

The different solvent extracts of the leaves of *Coffea canephora* and *Gravilea robusta* of Kolli hills were studied for DPPH (2, 2 diphenyl-1-picryl hydrazyl) free radical scavenging activity. Extracts of the concentrations of 10, 20, 30, 40 and 50 mg/mL were taken in small test tubes. The extracts were made up to 1 mL using methanol and 1 mL of 0.01 mM DPPH was added to each of the tube. Butylated Hydroxyanisole (BHA) was used as the reference and methanol as the blank. After half an hour of incubation in dark at room temperature, the absorbance was read at 517 nm. The percent inhibition was calculated using the formula:

$$\text{Effective concentration} \% = \left(\frac{\text{Control Absorbance} - \text{Test Absorbance}}{\text{Control Absorbance}}\right) \times 100$$

The Effective Concentration (EC50) that inhibits the formation of DPPH radicals by 50% is reported (Bhuvaneswari et al., 2014 and Udayaprakash et al., 2014b).

Larvicidal activity

The eggs of *Artemia salina* were procured from Philadelphia, USA. In a small water tank containing sea water, the eggs were incubated for 48 hours for hatching. Required light was provided with Philips 40 Watts lamp for 12 h cycle. After 48 h, the larvae were used for the experiments. The naupliii of *Artemia salina* were challenged in different test tubes containing 10 mL of sea water and 20 larvae. To this, extracts of leaves at different concentrations (20, 40, 60, 80 and 100 µg/mL) were added. The mortality of the larvae was observed at 24 and 48 h (Udayaprakash et al., 2011 and 2012).

Nauplii were considered dead when they were immobile and stayed at the bottom of the test tubes. The percent mortality of brine shrimp was calculated as:

$$\% \text{Mortality} = \left(\frac{\text{No. of brine shrimp dead}}{\text{No. of brine shrimp introduced}}\right) \times 100$$

Pesticidal activity
The adult pests of *Sitophilus oryzae* were collected from naturally infested rice grains supplied through Public Distribution System of Chennai, Tamil Nadu. The pests were reared in the laboratory, in plastic containers with fresh rice grains. The containers were covered with muslin cloth to allow sufficient ventilation.

Leaf extracts of 0.5 mL, 1 mL and 1.5 mL volume constituting 50 mg, 100 mg and 150 mg concentration respectively were poured into a clean Petri plate and allowed to dry. A plug of cotton was used to wipe the extract from the plate and placed in a Petridish containing adult pests (20 in number) along with one gram of rice and sealed. The mortality rate of the rice weevil was observed after 24 h and 48 h of incubation (Udayaprakash et al., 2013d and e).

**RESULTS**

**Phytochemistry**

The phytochemical evaluation showed the presence of Tannin and steroid in the plant *Coffea canephora*; flavanoid and steroid in *Gravillea robusta*. All other phytochemicals, i.e. saponins, cardiac glycosides and phlobatannins were not recorded in either of the plants studied (Table 1).

**Antibacterial efficacy**

The antibacterial efficacy of acetone, chloroform, ethyl acetate and methanol extracts of the leaves of *Coffea canephora* and *Gravillea robusta* were evaluated by Micro broth dilution. The results indicated that the methanolic extract of *Coffea canephora* showed better antibacterial activity against the bacteria *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia* while the Ethyl acetate extract of *Coffea canephora* acted better against *Vibrio parahaemolyticus*. The results of MIC recorded for each solvent extract of the plants against the bacteria studied is presented in Table 2.

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<th>Table 1: Presence of phytochemicals in <em>Coffea canephora</em> and <em>Gravillea robusta</em> from Kolli Hills</th>
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<td>Phlobatannins</td>
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<th>Table 2: Minimum Inhibitory Concentration (MIC in µg/ml) recorded for the leaves of <em>Coffea canephora</em> and <em>Gravillea robusta</em> from Kolli Hills</th>
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<th>Table 3: EC50 value recorded for different solvent extracts of the leaves from Kolli Hills</th>
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Antioxidant ability

The acetone extract of Coffea canephora recorded the lowest EC$_{50}$ value at 10 mg/mL concentration. The plant Coffea canephora showed better antioxidant ability when compared to Gravillea robusta. The results of the percent inhibition of DPPH at different concentration by different solvent extracts are tabulated in Table 3.

Larvicidal and Pesticidal activity

The results showed 100% mortality rate of the larvae of Artemia salina at the lowest concentration of 20 μg of each extract of the plants, Coffea canephora and Gravillea robusta and the storage pest, Sitophilus oryzae at the concentration of 50 mg/mL.

DISCUSSION

The study reveals the need to evaluate other plant parts for their bioefficacy apart from the industrially used parts of the plant. The results clearly demonstrate that the leaves of Coffea robusta possesses potential antibacterial activity and also acts as a potential pesticidal agent. Thus, incorporation of the extract of the leaves of Coffea canephora in developing tooth paste to control oral bacteria can also be of industrial importance. However, the knowledge on validation and analytical methods is the need of the hour in applying these benefits for different applications (Usher, 2000).

Although many reports and review are available on the chemical constituents of coffee (Farah, 2012), they are confined to the seeds, either raw or roasted. The studies on the chemical composition of the leaves of the plant are also stressed.

Similarly, the application of the plant Gravillea robusta only as a shade tree and timber needs to be revisited. The methanolic extract of the plant leaf was reported to be anticarcinogenic. Chuang et al., (2011) has reported significant cytotoxicity against human breast (MCF-7), lung (NCI-H460), and central nervous system (SF-268) cell lines. The chemical constituents of the plant leaf was studied by different authors (Chuang and Hu, 2007; Yamashita et al., 2008 and 2010; Yukiko et al., 2012). Similar to Coffea canephora, the present study also reveals the pesticidal activity of the leaves of Gravillea robusta. Thus, conducting study on the application of different plant parts of the industrially important plant is emphasized.

CONCLUSION

Coffea canephora and Gravillea robusta from Kolli Hills were studied for their bioefficacy. The study suggests the plant Coffea canephora as a potential member against bacteria. The study also suggests that the plants studied can be utilized in controlling the storage pest, Sitophilus oryzae.

CONFLICT OF INTEREST

No Conflict of Interest lies between Authors.

REFERENCES


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