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Antibacterial and Antioxidant Activity of *Coffea benghalensis* Roxb.Ex Schult. Fruit against Human Bacteria

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ABSTRACT

Aqueous and solvent extract of fruit of Coffea benghalensis were tested for antibacterial activity against six human bacterial species and antioxidant activity in vitro condition. Among the six bacterial species tested, in aqueous extract Proteus vulgaris recorded a maximum inhibition of 28.0mm at 50µl concentration and least activity was observed in Escherichia coli and recorded 15.0mm inhibition at 50µl concentration. In solvent extract, petroleum ether showed a significant activity against all the test bacterial species and Salmonella typhimurium recorded a maximum antibacterial activity of 31.0mm at 50µl concentration. Petroleum ether extract was followed by chloroform and methanol extract. No activity was observed in Benzene and Ethanol extract. All the results obtained were compared to standard antibiotic Ampicillin and Methicillin. In antioxidant activity, Alcohol water extract recorded a maximum radical scavenging activity of 68.3% at 25µg concentration followed by alcohol extract, Hexane extract, water extract and chloroform extract. Compared to standard antioxidants, ascorbic acid, BHA, and alfa-Tocopherol, fruit extract of C. benghalensis also showed better antioxidant activity.
Keywords: Coffea benghalensis, Antibacterial, Antioxidant, Synthetic antibiotics.

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INTRODUCTION

Infections due to bacterial species remain a serious therapeutic problem. Emerging resistance of these species is seriously decreasing the number of effective antimicrobials [1]. In recent years, multiple drug resistance in both human and plant pathogens has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases [2]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [3]. To overcome this disadvantage the use of medicinal plants to treat human diseases has its roots in pre-historical times. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries [4]. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country [3]**.** Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world [5]. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [6]**.** Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide and hydroxyl, nitric oxide and peroxynitrite radicals play an important role in oxidative stress related to the pathogenesis of various important diseases[7]. In recent years, phytochemicals in medicinal plants have received a great deal of attention mainly on their role in preventing diseases caused as a result of oxidative stress which releases reactive oxygen species such as singlet oxygen and various radicals as a damaging side effect of aerobic metabolism[8]. In the present investigation, aqueous and solvent extract of fruits of Coffea benghalensis Roxb.ex Schult. belongs to family Rubiaceae were tested for antibacterial and antioxidant activity in vitro condition which is an ecofriendly approach.

MATERIALS AND METHODS

Plant material:

Fresh fruits of Coffea benghalensis Roxb.ex Schult. free from diseases were collected from Nagaon, Assam. The fruits were washed thoroughly 2-3 times with running water and once with sterile distilled water. Fruit material was then air dried on a sterile blotter under shade and used for extraction**.**

Extraction

Aqueous extraction:

Fifty grams of thoroughly washed fruits of C. benghalensis were macerated with 50ml of sterile distilled water in a waring blender (Waring International, New Hartford, CT, USA) for 10 minutes. The macerate was first filtered through double layered muslin cloth, and then centrifuged at 6000g for 30 minutes. The supernatant was filtered through Whatmann No.1 filter paper and sterilized at 120°C for 15 minutes. The extract was preserved aseptically in a brown bottle at 5°C until further use [9].

Solvent extraction:

Thoroughly washed fruits of C. benghalensis were dried until all the moisture content get evaporated, and then powdered with the help of Waring blender. 25 grams of shade dried powder was filled in the thimble and extracted successively with petroleum ether, benzene, chloroform, methanol and ethanol in a Soxhlet extractor for 48 hours. Solvent extracts were concentrated under reduced pressure and the extracts were preserved in airtight bottle until further use [10, 11]

Test Pathogens:

Four Gram negative bacteria viz., Escherichia coli, Proteus vulgaris, Salmonella typhimurium and Klebsiella pneumonia and two Gram positive bacteria viz., Streptococcus faecalis and Staphylococcus aureus collected from Research center, CMR Institute of Management Studies (Autonomous), Department of Biosciences, Kalyan nagar, Bangalore.

Antibacterial Assay

Preparation of standard culture inoculums of test organism:

Three or four colonies of the entire test Gram negative and Gram positive bacterial species were inoculated into 2 ml nutrient broth and incubated at 37^{o} C for 24 hours till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO.

Aqueous Extract

Agar cup diffusion method:

An overnight culture of E.Coli, P. vulgaris, S. typhimurium, K. pneumonia, S. faecalis and S. aureus was standardized to contain approximately 107cfu/ml and inoculated into 20 ml nutrient broth. The culture medium was allowed to set. Thereafter, all the inoculums were

swabbed over the surface of nutrient agar medium plate using sterile cotton swab. Using a sterile cork borer of 5 mm diameter, five wells were made in solidified sterile nutrient agar medium, one in the centre and four wells at the corner. The agar plugs were removed with a flamed and cooled wire loop. Then 10,20,30,40 and 50µl of aqueous extract of C. benghalensis fruit were placed in the wells made in inoculated plates. The treatment also includes 50 µl of sterilized distilled water as control. All the plates were incubated for 24 hours at 37^{o}C and zone of inhibition if any around the well were measured in millimeter (mm). For each treatment five replicates were maintained. The same procedure were followed for standard antibiotics Ampicillin (25mg) and Methicillin (25mg) to compare the efficacy of plant extract against test organisms[3].

Solvent Extract:

One gram of different solvent extract of C. benghalensis fruit were dissolved in 9 ml of methanol. The sterile nutrient agar medium in petri dishes was uniformly smeared with test cultures. 5 mm wells were made in each petri dish to which 10,20,30,40 and 50 µl of different solvent extracts dissolved in methanol were added. For each treatment ten replicates were maintained. Respective solvents served as control. Standard antibiotics viz., Ampicillin (25mg) and Methicillin (25mg) was used to compare the efficacy of solvent extract against test organisms [12].

Antioxidant assay:

Preparation of fruit Extract of C. benghalensis:

One gram of fruit powder of C. benghalensis was macerated with 50 ml of 60°C hot water and alcohol (1:1), alcohol, hexane and chloroform (1:1:1) using pestle and mortar. The resultant solutions were made up to 100ml with respective solvent or solvent mixture and kept overnight at 40^{o} C. The obtained suspension was centrifuged at 10,000 rpm for10 min at 4°C. The residue was again extracted with additional 50ml of solvent or solvent mixture. The respective supernatants were combined and filtered in Whatmann No. I filter paper and passed through microbial filter (0.045µm) and the volume of filtrate were noted. The water extract was lyophilized at -37°C and referred to as WECb (Water Extract of fruits of C. benghalensis). The alcohol-water (1:1) extract was evaporated at 40°C using rotary flash evaporator and freeze dried to obtain brown residue and is referred as AWECb (Alcohol Water (1:1) Extract of fruits of C. benghalensis). Similarly, alcohol extract, hexane extract and chloroform extract were concentrated separately under vacuum using rotary evaporator to a brown residue and the resulting material obtained were designated as AECb (Alcohol Extract of fruits of C. benghalensis), HECb (Hexane Extract of fruits of C. benghalensis) and CECb (Chloroform Extract of fruits of C. benghalensis). 10mg of each dried extract was dissolved in 0.1ml of respective extracting solvent or solvent mixture and made up to 10ml with water and mixed properly. The solution was filtered in 0.45µm microbial filter and stored at-200C for further studies. The standard antioxidants were used at the concentrations based on the literature [13].

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1.1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Effect:

DPPH radical scavenging activity [14] of WECb, AECb, AWECb, HECb, CECb and ascorbic acid were assessed at various concentrations ranging from 10-100µg and were mixed in 1ml of freshly prepared 0.5mM DPPH ethanol solution and 2ml of 0.1M acetate buffer (pH 5.5). The resulting solutions were then left to stand at 37°C for 30 minutes prior to being spectrophotometrically detection at 517ηm. Standard antioxidants such as BHA and L- ascorbic acid were used to determine the radical scavenging activity under the same assay conditions. Lower absorbance at 517ηm represents higher DPPH scavenging activity. Blank test or control received the appropriate quantity of the solvent without any inhibitor or test sample. The percent inhibition was calculated from the following equation.

$$\text{Percent DPPH radical scavenging activity} = \frac{\text{OD of control-OD of test sample}}{\text{OD of control}} \times 100$$

RESULTS

Aqueous extract: Among the six bacterial species tested P. vulgaris recorded a maximum inhibition of 28.0mm at 50µl concentration and 5.0mm inhibition at 10µl concentration. P.vulgaris is followed by S.typhimurium and recorded 24.0mm inhibition. S.aureus showed moderate activity of 23.0mm inhibition and K.pneumonia and S.faecalis recorded 21.0mm and 21.0mm inhibition at 50µl concentration tested. Least inhibition was observed in E.Coli (15.0mm) at 50µl concentration. Significant activity was also observed in 20, 30 and 40µl concentration against all the bacterial species tested (Table1).

Table1: Antibacterial activity of aqueous extract of C. benghalensis (Fruit)

Bacteria	Zone of Inhibition(mm)						
	Concentration						
	10µl	20µl	30µl	40µl	50µl	Ampicillin (25mg)	Methicillin (25mg)
E.Coli	5.0^{a} ±0.1	6.0^{b} ±0.0	8.8^{c} ±0.0	11.0^{d} ±0.0	15.0^{e} ±0.0	28.0^{a} ±0.0	33.0^{b} ±0.0
P.vulgaris	8.0^{a} ±0.0	14.0^{b} ±0.1	17.0^{c} ±0.1	23.0^{d} ±0.1	28.0^{e} ±0.1	33.0^{a} ±0.0	33.0^{a} ±0.0
S. typhimurium	7.0^{a} ±0.1	12.0^{b} ±0.3	15.0^{c} ±0.0	19.0^{d} ±0.2	24.0^{e} ±0.2	34.0^{b} ±0.0	31.0^{a} ±0.0
K. pneumonia	6.0^{a} ±0.0	11.0^{b} ±0.0	14.0^{c} ±0.1	18.0^{d} ±0.1	21.0^{e} ±0.0	33.0^{b} ±0.1	32.0^{a} ±0.0
S. faecalis	4.0^{a} ±0.2	8.0^{b} ±0.1	13.0^{c} ±0.2	17.0^{d} ±0.0	21.0^{e} ±0.2	22.0^{a} ±0.0	33.0^{b} ±0.0
S. aureus	6.0^{a} ±0.0	13.0^{b} ±0.2	16.0^{c} ±0.0	19.0^{d} ±0.0	23.0^{e} ±0.0	32.0^{a} ±0.0	32.0^{a} ±0.0

- Values are the mean of three replicates, ± standard error.
- The means followed by the same letter (S) are not significantly different at P<0.05 when subjected to Tukey's HSD.
- Pattern of percent Inhibition increase is not uniform for all the microorganisms.

Compared to standard antibiotics, In Ampicillin, maximum inhibition was observed in S.typhimurium (34.0mm). Significant inhibition was observed in P.vulgaris (33.0mm), K.pneumonia (33.3mm), S.aureus (32.0mm), E.Coli (28.0mm) and S.faecalis (22.0mm) at recommended dosage of 25µg concentration (Table1).

In Methicillin, E.Coli, P.vulgaris and S.faecalis recorded 33.0, 33.0 and 33.0mm inhibition followed by K.pneumonia (32.0mm), S.aureus (32.0mm) and S.typhimurium (31.0mm) at 25µg of recommended concentration (Table1).

Solvent extract: Among the five solvent extracts tested at 10,20, 30, 40 and 50µl concentration against six bacterial species, in Petroleum ether extract S.typhimurium recorded a maximum inhibition of 31.0mm at 50µl concentration followed by P.vulgaris (30.0mm), S.aureus (29.0mm), K.pneumonia (28.0mm), E.Coli (27.0mm) and S.faecalis (25.0mm) respectively. Significant activity was also observed in 10µl to 40µl concentration against all the test bacterial species (Table 2). No activity was observed in benzene extract tested against all the six bacterial species. In chloroform extract, E.Coli and S.aureus recorded 19.0mm and 19.0mm inhibition at 50µl concentration. S.faecalis recorded 17.0mm and K.pneumonia recorded 14.0mm inhibition at 50µl concentration tested. No activity was observed in P.vulgaris and S.typhimurium at different concentration (Table 2). In Methanol extract, E.Coli recorded maximum of 21.0mm inhibition followed by S.aureus (19.0mm), P.vulgaris (17.0mm), K.pneumonia (16.0mm), S.typhimurium (15.0mm) and S.faecalis (15.0mm) inhibition respectively at 50µl concentration. No activity was observed in ethanol extract in different concentration tested against all the test bacterial species(Table 3).

Antioxidant assay

1.1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Effect: Alcohol water extract recorded a maximum DPPH radical scavenging activity of 68.3 percent at 25 µg concentration followed by alcohol extract and recorded 55.5 percent at 100 µg concentration. In hexane extract, at 100 µg concentration significant radical scavenging activity was recorded (45.5%). In water extract, 43.5% of antioxidant was observed at 100 µg concentration. Least activity was observed in chloroform extract and recorded 41.1% antioxidant activity at 100 µg concentration. Compared to standard neutraceutical antioxidants such as ascorbic acid showed 82.2% activity at 100 µg concentration , BHA recorded 86.0% radical scavenging activity at 72µg, alfa-Tocopherol recorded 76.3% activity at 80.0 µg concentration(Table 4).

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Table 4: 1.1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging activity of extracts of C. benghalensis (Fruit).

Antioxidant	Activity Concentration	% DPPH Radical Scavenging activity
Control	No antioxidant	0.0
Water Extract	100 µg	43.5
Alcohol extract	100 µg	55.5
Alcohol water(1:1) extract	25 µg	68.3
Hexane extract	100 µg	45.5
Chloroform extract	100 µg	41.6
Ascorbic acid	100 µg	82.3
BHA	72.0 µg	86.0
alfa-Tocopherol	80.0 µg	76.3

DISCUSSION

Diseases caused by bacteria are widespread worldwide. The treatment of these infections is mainly based on the use of antibiotics. In recent years, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes [15]. To avoid the use of synthetic antibiotics, the herbal medicine has recently been used in the United Nations and other developing countries like India as an alternative to conventional medicine. In many parts of the world, medicinal plants have continued to be an integral part of the health care system and the people's culture. Traditional medical treatments in daily life are now being used with empirical methods [16]. In the present investigation aqueous extract and solvent extract (Petroleum ether, chloroform and methanol) recorded a strong antibacterial activity against P.vulgaris, S.typhimurium and K.pneumonia compared to synthetic antibiotics Ampicillin and Methicillin. The antioxidant activity of fruit extract was also significant and the activity was near to standard antibiotics. Alcohol water extract recorded a strong antioxidant activity at lower 25µg concentration. Hence an identification of bioactive compound is necessary in alcohol extract.

CONCLUSION

From the result and observation, aqueous and solvent extract of fruit of C. benghalensis showed strong antibacterial and antioxidant activity. Hence a further isolation of bioactive compound and purification is necessary in petroleum ether extract which have recorded a maximum activity compared to other solvent extracts. Also a further identification of bioactive compound in different solvent extract is necessary which have recorded strong antioxidant activity.

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Table 2: Antibacterial activity of solvent extracts of C. benghalensis (Fruit).

Bacteria	Zone of Inhibition(mm)																
	Concentration																
	Petroleum ether extract					Benzene Extract					Chloroform extract					Ampicillin	Methicillin
	10µl	20µl	30µl	40µl	50µl	10µl	20µl	30µl	40µl	50µl	10µl	20µl	30µl	40µl	50µl	(25mg)	(25mg)
E.Coli	6.0^{a} ±0.0	13.0^{b} ±0.0	18.0^{c} ±0.0	22.0^{d} ±0.0	27.0^{e} ±0.0	-	-	-	-	-	4.0^{a} ±0.0	9.0^{b} ±0.0	13.0^{c} ±0.2	16.0^{d} ±0.0	19.0^{e} ±0.0	28.0^{a} ±0.0	33.0^{b} ±0.0
P.vulgaris	9.0^{a} ±0.0	15.0^{b} ±0.0	19.0^{c} ±0.0	23.0^{d} ±0.1	30.0^{e} ±0.1	-	-	-	-	-	-	-	-	-	-	33.0^{a} ±0.0	33.0^{a} ±0.0
S. typhimurium	10.0^{a} ±0.1	17.0^{b} ±0.1	21.0^{c} ±0.1	25.0^{d} ±0.2	31.0^{e} ±0.2	-	-	-	-	-	-	-	-	-	-	34.0^{b} ±0.0	31.0^{a} ±0.0
K. pneumonia	8.0^{a} ±0.0	14.0^{b} ±0.2	17.0^{c} ±0.2	21.0^{d} ±0.0	28.0^{e} ±0.0	-	-	-	-	-	2.0^{a} ±0.1	5.0^{b} ±0.1	8.0^{c} ±0.0	10.0^{d} ±0.0	14.0^{e} ±0.1	33.0^{b} ±0.1	32.0^{a} ±0.0
S. faecalis	7.0^{a} ±0.1	13.0^{b} ±0.1	16.0^{c} ±0.0	20.0^{d} ±0.1	25.0^{e} ±0.0	-	-	-	-	-	3.0^{a} ±0.3	7.0^{b} ±0.2	12.0^{c} ±0.0	14.0^{d} ±0.1	17.0^{e} ±0.0	22.0^{a} ±0.0	33.0^{b} ±0.0
S. aureus	8.0^{a} ±0.2	15.0^{b} ±0.0	18.0^{c} ±0.2	21.0^{d} ±0.2	29.0^{e} ±0.0	-	-	-	-	-	4.0^{a} ±0.2	8.0^{b} ±0.3	14.0^{c} ±0.0	15.0^{d} ±0.0	19.0^{e} ±0.2	32.0^{a} ±0.0	32.0^{a} ±0.0

- Values are the mean of three replicates, ± standard error.
- The means followed by the same letter (S) are not significantly different at P<0.05 when subjected to Tukey's HSD.
- Pattern of percent Inhibition increase is not uniform for all the microorganisms.

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Table 3: Antibacterial activity of solvent extracts of C. benghalensis (Fruit).

Bacteria	Zone of Inhibition(mm)											
	Concentration											
	Methanol Extract					Ethanol Extract					Ampicillin	Methicillin
	10µl	20µl	30µl	40µl	50µl	10µl	20µl	30µl	40µl	50µl	(25mg)	(25mg)
E.Coli	5.0 ±0.0	9.0 ±0.0	13.0 ±0.0	16.0 ±0.2	21.0 ±0.0	-	-	-	-	-	28.0[a] ±0.0	33.0[b] ±0.0
P.vulgaris	4.0 ±0.0	9.0 ±0.1	12.0 ±0.1	15.0 ±0.1	17.0 ±0.0	-	-	-	-	-	33.0[a] ±0.0	33.0[a] ±0.0
S. typhimurium	2.0 ±0.1	7.0 ±0.2	10.0 ±0.2	13.0 ±0.0	15.0 ±0.1	-	-	-	-	-	34.0[b] ±0.0	31.0[a] ±0.0
K. pneumonia	4.0 ±0.0	8.0 ±0.0	12.0 ±0.0	14.0 ±0.0	16.0 ±0.0	-	-	-	-	-	33.0[b] ±0.1	32.0[a] ±0.0
S. faecalis	2.0 ±0.2	7.0 ±0.0	10.0 ±0.3	13.0 ±0.2	15.0 ±0.0	-	-	-	-	-	22.0[a] ±0.0	33.0[b] ±0.0
S. aureus	4.0 ±0.0	8.0 ±0.0	13.0 ±0.2	16.0 ±0.1	19.0 ±0.0	-	-	-	-	-	32.0[a] ±0.0	32.0[a] ±0.0

- Values are the mean of three replicates, ± standard error.
- The means followed by the same letter (S) are not significantly different at P<0.05 when subjected to Tukey's HSD.
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REFERENCES

[1] Nychas GJE. Natural antimicrobials from plants. In: Gould GW (Ed) New Methods of Food Preservation, Blackie Academic Professional, London 1995: 58-89.
[2] Jamuna BA, Ravishankar RV, Pradeepa VS. Malaysian J Microbiol 2011; 7(1):14-18.
[3] Joshi B, Lekhak S, Sharma A. Kathmandu University Journal of Science, Engineering and Technology 2009; 5(1): 143-150.
[4] Kamali HHEL , Mohammed YELA. Curr Res J Biol Sci 2010; 2(2): 143-146.
[5] Ali MA, Mozid MA, Yeasmin MS, Khan AM, Sayeed MA. Res J Agr Biol Sci 2008; 4(6): 871-874.
[6] Souri E, Amin G, Farsam H, Jalalizadeh A, Barezi S. Iranian J Pharm Res 2008;7 (2): 149-154.
[7] Shyur LF, Tsung JH, Chen JH, Chiu CY, Lo CP. International Journal of Applied Science and Engineering 2005; 3(3): 195-202.
[8] Balaji R , Prakash G , Suganya DP , Aravinthan KM. International Journal of Pharma, Research and Development 2011;3(1): 20-27.
[9] Verma S, Dohroo NP. Plant Dis Res 2003; 18(2):131–134.
[10] Nostro A, Germano MP, Angelo VD, Marino A, Cannatelli MA. Planta Med 2000; 25:20-24.
[11] Srinivasan D, Nathan S, Suresh T, Lakshmanaperumalsamy P. Asian J Microbiol Biotechnol Env Sci. 2001;3(1-2):67–70.
[12] Lalitha V, Kiran B, Raveesha KA. Pharmacophore 2011;2(1): 78-85.
[13] Kiran B, Raveesha KA. World Applied Sciences Journal 2010; 8(8): 985-990.
[14] Benguo L, Zhengxiang N, Vu Z, Keyong X , Jianhua G. J Food Chemist 2008;32(4): 431-442.
[15] Rajesh K, Harsha R, Ahmed MG, Hareesh AR, Thammanna GSS, Dinesha R, Satish KBP, Irfan AM. Res J Pharm Biol Chem Sci 2010;1(3): 691-698.
[16] Ramya DK, Karthikumar S , Jegatheesan K. African J Plant Sci 2010;4(5): 163-166.