Research Article

Anti-tubercular and antioxidant screening of *Annona Reticulata* Linn. and *Borassus Flabellifer* Linn.

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Abstract

Background: The *Borassus flabellifer* Linn. and *Annona reticulate* Linn. plant parts such as leaves, fruit, bark, and seed have been reported for possessing anti-inflammatory, antiarthritic activity, and antibacterial activity. This work was, therefore, designed to examine an antioxidant and antimicrobial activities of methanol extract and chloroform, acetone, and n-butanol fractions. **Materials and Methods:** Antioxidant activity was determined by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH), H_2O_2, radical scavenging assay, total antioxidant activity, and $Fe3^+$ reducing power assay. The anti-tubercular activity was investigated by both the agar well and disk diffusion method by three strains of mycobacteria, MIC. **Results:** Antioxidant activity of methanol extract, chloroform fractions, n-butanol fraction, acetone fraction of *Borassus flabellifer* Linn., and methanolic extract of *Annona reticulate* Linn. were found to inhibit DPPH and hydrogen peroxide free radicals as 40–59%, 45–60%, 47–68%, and 39–65%, for methanolic extract of *Annona reticulate* Linn. and 48–68%, 28–61%, 43–75%, 31–46%, and 37–80% for methanolic extract of *Annona reticulate* Linn. 12–75%. While, total antioxidant result was found between 1% and 33%, 1% and 40%, 2% and 42%, and 16% and 47% for methanolic extract of *Annona reticulate* Linn. 9%–17%. Potential of methanol extract and fractions for reducing of $Fe3^+$ was found fairly moderate when compared with ascorbic acid as standard. **Conclusion:** Results clearly reveal that the extract and fractions is found to possess considerable significant activity against mycobacterial infections and found to have deteriorating potential in oxidative damage induced by free radicals to the cell and cell components.

Keywords: Antioxidant, anti-tubercular, 1, 1-diphenyl-2-picrylhydrazyl, minimum inhibitory concentration

Introduction

Infectious diseases are the major cause of morbidity and mortality and thus a serious public health problem in developing countries.[1] Medicinal plants indicate rich source of antimicrobial agents. Number of the plants materials used in traditional medicine is easily available in rural areas at comparatively economical than modern medicine.[2] Antimicrobial agents are essentially important in reducing the global burden of infectious diseases.[3] Essential oils derived from medicinal herbs have versatile applications in ethno-medicine, cosmetics, food, beverages, preservation, fragrance, and pharmaceutical industries. These bioactive compounds indicate several positive biological properties, such as antioxidant, antiviral, antibacterial, antifungal, insecticidal, and anticancer activities.[4] The arrival of organisms resistant to nearly all classes of antimicrobial agents becomes a serious public health concern in the past several years. The plants that exhibit great activity might be considered as a source of potential antimicrobial compounds.[5] Tuberculosis (TB) is a disease that causes a high mortality every year. *Mycobacterium tuberculosis*, the etiological agent of TB, is responsible for life-threatening intracellular pathogen in humans. It infects approximately one-third of the world's population. This can result in 2–3 million deaths. Global prospects for TB control are challenged by the emergence of drug-resistant strains. If we are to stay ahead of mycobacterial strains developing resistance to chemotherapeutic regimens, all new agent leads must be explored.[6] Antimicrobial and antioxidant effects of Asteraceae species, applications of these extracts in treating infectious diseases need an evaluation of pathogenic bacterial strains isolated from clinical specimens.[7,8]

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Conventionally *Borassus flabellifer* Linn.is called Palmyra palm which is immensely distrusted in tropical regions of the Asian continent. Appreciable noteworthy economic value to the local population is aided by the *Borassus flabellifer* Linn. Seed coat extract of the *Borassus flabellifer* shows antimicrobial activity.[9] Male inflorescence shows significant anti-inflammatory activity.[10] Almost all part of palm is used as medicinal use. The different parts of plant are being used for medicinal properties such as anthelmintic,[11] diuretics, antioxidant activity of the fruits, bacterial activity of the fruits, wound healing, immunomodulatory, and antimalarial. Hence, in the current investigation, we are concentrating over the antioxidant and antimicrobial activity of extract and fraction, respectively.[8,12]

The common name of *Annona Reticulata* is Ramphal belonging to family Annonaceae.[13] Conventionally, the plant is used for antibacterial infection, epilepsy, dysentery, cardiac problem, worm infestation, constipation, hemorrhage, dysuria, fever, and ulcer also it is used as antiparasitic, insecticide, antidysenteric, and antidiarrhoeic.[13,14] The bark is a powerful astringent and used as a tonic and the leaves are used in the anthelmintic treatment. All the parts of plant such as root, leaves, fruit, and bark are medicinally useful. From the literature survey, we found that the various activities were reported on plant such as antipyretic, anthelmintic, anti-hyperglycemic, *in vitro* and *in vivo* cytotoxic, recombinant caspase inhibitory activity, analgesic, and anti-inflammatory.[13,15,16] Plant parts are reach in steroidal saponin, flavonoids, tannins, glycoside, alkaloid contents and a mitochondrial complex inhibitor, and acetogenin.[14,16] Hence, it is needed to reveal traditional claims of plant and phytoconstituents for antimicrobial activity.[16]

Free radical is produced in normal or pathological cell metabolism. Oxidation reactions, often radical initiated, are imperative processes in biological system.[17] Numerous reasons can be allocated to validate its importance: Knowledge about reactive oxygen and nitrogen species invention and metabolism; identification of biomarkers for oxidative damage; evidence relating manifestation of chronic and some acute health difficulties to oxidative stress; and identification of several dietary antioxidants presents in plant foods as bioactive molecules.[18,19] The word itself is magic using the oxidant concept as a spearhead in projected mechanism for staving off so-called "free-radical." The rush is onto mine claims for the latest and most effective combination of free-radical scavenging compound.[18,20]

Materials and Methods

Collection of extracts

Borassus flabellifer and *Annona reticulate* Linn plant extracts were collected from Vikas Suryawanshi and Amruta Wattamwar, respectively, research students (2013) at School of Pharmacy S R T M U, Nanded.

Reagents, chemicals, and microorganism

Methanol, dimethyl sulfoxide, sodium hydroxide sodium phosphate, and Potassium ferricyanide were purchased from Rankem (India). Ascorbic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), ammonium molybdate, and ferric chloride were obtained from HiMedia. Potassium dihydrogen phosphate (S. D. Fine, Mumbai) was purchased from respective vendors.

Microorganism used for anti-tubercular study has been collected form Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh [Table 1].

Antioxidant activity

DPPH free radical scavenging assay

The ability of the extract to scavenge the DPPH free-radical was assayed according to the method modified from Manssouri *et al*. and Ali *et al*. and Sun *et al*.[21-23] 0.1 nM DPPH solution was prepared by addition of methanol. 1.9 mg of DPPH in methanol and volume was made up to 100 ml with methanol. The mixture was shaken vigorously and solution was kept in dark condition for 30 min to complete reaction. 1 ml of DPPH solution was added to 1 ml of different (20, 40, 60, 80, and 100 mg/ml) concentrations of extract and allowed to stand at room temperature for 30 min. The mixture was measured spectrophotometrically (UV-1800, UV-VIS Spectrophotometer, Shimadzu) at 517 nm. DPPH radical scavenging activity (%) of the sample was calculate as following formula.

$$\text{Radical scavenging activity}(\%) = 1 - \frac{A(\text{sample})}{A(\text{control})} \times 100$$

Where, A control is the absorbance of the control and sample is the absorbance of the extract or standard. For standard of ascorbic acid was a run using same concentration as that of extract.[21-24]

Hydroxyl (H_2O_2) radical scavenging activity assay

The ability of the extract sample to scavenge H_2O_2 was determined according to the method of Ali *et al*. and Jamkhande *et al*.[22,25] Solution of 0.2 M potassium dihydrogen phosphate and 0.2 M sodium hydroxide solutions was prepared as per the Indian Pharmacopoeia 1996 standards. 50 ml potassium dihydrogen phosphate solution was placed in a 200 ml volumetric flask and 39.1 ml of 0.2 M sodium hydroxide solution was added and finally volume was made up to 200 ml with distilled water to prepare phosphate buffer (pH-7.4). 50 ml of phosphate buffer solution was added to equal amount of hydrogen peroxide to generate the free radicals and solution was kept aside at room temperature for 5 min to complete the reaction. Extracts (1 ml) in distilled water were added to 0.6 ml hydrogen peroxide solution and the absorbance was measured at 230 nm in a spectrophotometer (UV-1800, UV-VIS spectrophotometer, Shimadzu) against a blank solution containing phosphate buffer solution without hydrogen peroxide. Concentrations selected for extract were ranging from 20 to 100 mg/ml. The percentage of scavenging of H_2O_2 of extract was measured using the following equation:

Table 1: Bacterial microorganisms and their maintenance

Bacteria	MTCC No.	Temperature required for the growth of bacteria (°C)	Incubation period
M. Smegmatis	994	37°C	1 day
M. Tuberculosis	300	37°C	3 weeks
M. Phlei	1724	37°C	5 days

Hydrogen Peroxide % scavenging activity [H_2O_2] = (A0-A1)/A0 × 100

Where, A0 is the absorbance of the control, and A1 is the absorbance in the presence of the extract sample.[22,25,26]

Total antioxidant capacity assay

Total antioxidant capacities of the extract samples and ascorbic acid were determined according to the method.[22,24] Different concentrations (10, 20, 40, 60, 80, and 100 μg/ml) of sample solutions were prepared from the stock solution in DMSO. Reagent solution was prepared (Mix 0.6 M Sulfuric acid + 28 mM sodium phosphate + 4 mM ammonium molybdate). An aliquot (0.1 ml) of these fractions and the standard (ascorbic acid), respectively, combined with 1 ml of reagent solution. Reaction mixture was incubated at 95°C for 90 min. After the samples were cooled to 25°C, the absorbance was measured at 695 nm against a blank. The control contained all reagents except the extract fraction while DMSO was used as a blank. The total antioxidant activity was expressed as the absorbance value at 695 nm. All tests were run in duplicate and analyses of the samples were run in triplicate and averaged. The higher absorbance value indicates the greater antioxidant activity.[22,24]

Fe^{3+} reducing power assay

Reducing power assay of extract determined by Ali *et al.* and Sun *et al*.[22,23] Which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferricyanide (Fe^{2+}), which reacts with ferric chloride to form ferric-ferrous complex that has absorption at 700 nm. The antioxidants presented in the test solution can reduce the ferricyanide complex to the ferrous form by donating an electron. The reducing power can be measured by detecting the formation of Perl's Prussian blue at 700 nm. Varying concentrations of extract (50,100,150,200, and 250 μg) in double distilled water were mixed with 2.5 ml of phosphate buffer and were 2.5 ml mixed with 0.125 mL of potassium ferricyanide (1%, w/v) and incubated at 50°C for 20 min, 0.125 mL of trichloroacetic acid (10%, w/v) was added to the mixture to terminate the reaction. The solution was mixed with 1.5 ml of ferric chloride (0.1%, w/v), and the absorbance was measured at 700 nm. An increased absorbance of the reaction mixture indicated increased reducing power.[22,23,26]

Anti-tubercular screening activity

Agar cup well method

The agar cup well method was used for extracts and fractions were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 50 mg/ml to make a stock solution. The stock solution was sterilized using 0.2 μm single use filters. Sterile distilled water was used for further dilution. Water extracts were dissolved in sterile distilled water. A concentration of 50.0 μg per well was used for the general susceptibility tests. A stock solution of rifampicin, 3.0 mg/ml was prepared a concentration of 20.0 mg/ml. The medium for incubation was sterile Middle brook 7H11 agar in 90-mm diameter Petri dishes with quadrants. In each quadrant of the Petri dish was put 5.0 ml of the medium. The solidified medium in the quadrants was inoculated using the flooding method so that a uniform surface distribution of inoculum was obtained. Wells of 5.0 mm diameter and 2.5 mm depth were then bored in the dry inoculated medium using a sterile cork borer, and 50 μl of the test extract, were dispensed into the well of the first quadrant in each Petri dish giving an extract concentration of 50.0 μg per well. A volume of 50 μl of the 1.0 mg/ml solution of rifampicin and an equal volume of each of the plant extracts were dispensed into the well of the second quadrant giving a drug concentration of 1.0 μg per well. The well in the third quadrant was left empty as a control, while the well in the fourth quadrant was filled with the solvent used to dissolve the extract, also as a control. The Petri dishes were then left in the hood overnight to allow diffusion of the extracts and drug and then sealed with a carbon dioxide-permeable tape and incubated at 37°C.[4,27-29]

Disk diffusion method

The method described by .[4,30] The bacterial inoculum was prepared from overnight-grown cultures (24 h) in nutrient broth (Difco) containing tween-80 and the turbidity was adjusted equivalent to 0.5 McFarland units (approximately1.2*108 CFU ml^{-1}). Aliquots (100 μl) of inoculums were spread over the surface of agar plates with a sterile glass spreader. The paper disk (5 mm diameter, Whatman filter paper no. 3; Millipore) was impregnated with 10 μl of test sample and allowed to dry for 30 min and then placed on the premade bacterial lawn. The disks containing solvents served as negative control. The disk containing antibiotic rifampicin (5.0 μg $disk^{-1}$; Sigma, St Louis, MO) was used as positive control. The plates were then incubated for 48 h at 37°C, and the zone of bacterial growth inhibition around disk was measured. The assay was repeated twice, and mean of the three experiments was recorded.[4,30]

Minimum inhibitory concentration (MIC)

The MIC was determined by broth dilution method.[7,31] Each strain was tested with sample that was serially diluted in broth to obtain concentrations ranging from 0.09 to 9 mg/mL. The sample, previously sterilized with Millipore filter of 0.20 mm, was stirred and inoculated with 50 mL suspension of 106 CFU/mL of the tested microorganisms, and incubated for 24 h at 37°C for bacteria, at 28°C for yeasts and molds. Another culture medium without adding microorganism's suspension was prepared as the negative control. The MIC value was determined as the lowest concentration of the sample at which the tested microorganisms did not demonstrate any visible growth after incubation.[7,30,31]

Results

Antioxidant activity

DPPH assay

The antioxidant activities of methanol extract and fraction of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. were evaluated by the DPPH radical scavenging capacity. Table 1 shows the percentage of DPPH radicals scavenging capacity with ascorbic acid reference as standard. In the DPPH scavenging assay, the IC_{50} (the concentration required to scavenge 50% of radical) values of methanol extracts and fractions of plants and ascorbic acid were 57.14 μg/mL, 34.64 μg/mL, 32.47 μg/mL, 47.95 μg/mL, 29.42μg/mL, and 52.57 μg/mL, respectively. *P* value is $P < 0.0001$:***, $P < 0.01$:**, $P < 0.05$:* DPPH assay description is shown in Table 2.

H_2O_2 assay

The antioxidant activity of extract and fraction of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. were evaluated by the H_2O_2 radical scavenging capacity. Table 2 shows the percentage of H_2O_2 scavenging capacity with ascorbic acid reference as standard. In the H_2O_2 scavenging assay, the IC_{50} (the concentration required to scavenge 50% of radical) values of methanol extracts and fractions of plants and ascorbic acid were 229.03 µg/mL, 57.12 µg/Ml, 352.96 µg/mL, 484.92 µg/mL, 166.07µg/mL, and 85.03 µg/mL, respectively. *P* value is $P < 0.0001$:***, $P < 0.01$:**, $P < 0.05$:*H_2O_2 assay description shown in Table 3.

Total antioxidant activity

The antioxidant activity of extract and fractions of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. were evaluated by the total antioxidant capacity activity assay. Table 3 shows the percentage of total antioxidant capacity activity scavenging capacity with ascorbic acid reference as standard. In the total antioxidant capacity activity scavenging assay, the IC_{50} (the concentration required to scavenge 50% of radical) values of methanol extracts and fractions of plants and ascorbic acid were 166.43 µg/mL, 128.10 µg/mL, 135.57 µg/mL, 96.06 µg/mL, and 94.80 µg/mL, µg/mL respectively. *P* value is $P < 0.0001$:***, $P < 0.01$:**, $P < 0.05$:*Total antioxidant activity description is shown in Table 4.

Fe^{3+}reducing powers assay

The antioxidant activity of extract and fractions of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. were evaluated by the Fe^{3+} reducing power assay. Table 4 shows scavenging capacity of extracts that are compared with the ascorbic acid as standard. Reducing power of methanol extracts and fractions of plants and ascorbic acid reference as standard at 250

Table 2: Radical scavenging activity (Percent inhibition) of the extract and fraction of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. by the DPPH method

Concentration in (µg/ml)	V1 (%Inhibition)	V2 (%Inhibition)	V3 (%Inhibition)	V4 (%Inhibition)	A1 (%Inhibition)	A (%Inhibition)
20	40.54±0.57***	45.90±0.95***	47.79±0.54***	43.69±0.66***	48.10±0.63***	39.60±0.58***
40	44.95±1.22***	52.52±0.43***	50.94±1.20***	47.68±1.23***	53.15±0.24***	44.53±0.48***
60	51.78±0.91***	54.93±0.53***	57.56±0.92***	51.78±0.76***	61.44±0.56***	53.88±0.39***
80	56.51±0.75***	58.08±0.66***	59.34±0.45***	58.19±0.61***	65.54±0.91***	58.92±0.12***
100	59.76±1.01***	60.18±0.66***	68.17±0.09***	64.07±0.30***	68.06±0.46***	65.23±0.29 ***
IC_{50}	57.14±0.57***	34.64±0.95***	32.47±0.54***	47.95±0.66***	29.42±0.63***	52.57±0.58***

Values are expressed as mean±SEM (n=3). A: Ascorbic acid, V1: Methanol extract, V2: Chloroform extract, V3: N-butanol extract, V4: Acetone extract and A1: Methanol extract of *Annona reticulate* Linn.

Table 3: Radical scavenging activity (Percent inhibition) of the extract and fraction of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. by H_2O_2 assay.

Concentration in (µg/ml)	V1 (%Inhibition)	V2 (%Inhibition)	V3 (%Inhibition)	V4 (%Inhibition)	A 1 (%Inhibition)	A (%Inhibition)
50	28.04±0.29***	43.15±0.01***	31.16±0.34***	−33.56±0.52***	12.46±0.29***	37.67±0.014***
100	31.84±0.47***	62.84±0.02***	31.84±1.05***	−32.87±0.51***	31.84±0.41***	57.97±0.01***
150	32.19±0.20***	65.13±0.01***	35.27±0.40***	−30.13±0.13***	46.06±0.28***	63.83±0.017***
200	37.67±0.26***	66.19±0.20***	35.95±1.06***	−26.02±0.34***	59.58±0.34***	79.82±0.017***
250	61.98±0.26***	75.06±0.01***	46.23±0.21***	16.43±0.36***	75.34±0.25***	80.37±0.014***
IC_{50}	229.03±0.29***	57.12±0.01***	352.96±0.34***	484.92±0.52***	166.07±0.29***	85.03±0.014***

Values are expressed as mean±SEM (n=3). A: Ascorbic acid, V1: Methanol extract, V2: Chloroform extract, V3: N-butanol extract, V4: Acetone extract and A1: Methanol extract of *Annona reticulate* Linn.

Table 4: Radical scavenging activity (Percent inhibition) of the extract and fraction of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. by total antioxidant assay

Concentration in (µg/ml)	V1 (%Inhibition)	V2 (%Inhibition)	V3 (%Inhibition)	V4 (%Inhibition)	A1 (%Inhibition)	A (%Inhibition)
20	1.87±0.0010***	1.05±2.84***	2.08±0.001***	16.07±5.77***	9.61±0.0017***	20.33±0.0023***
40	3.68±0.0011***	4.66±1***	3.88±0.001***	29.85±0.01***	15.77±0.0046***	30.88±0.0029***
60	9.44±0.0011***	21.40±0.002***	9.44±0.002***	44.04±0.07***	16.22±0.0011***	32.214±0.062***
80	11.32±0.003***	20.20±0.057***	16.07±0.008***	36.48±0.04***	17.39±0.0011***	38.96±0.0057***
100	33.80±0.030***	40.12±0.006***	42.68±0.004***	47.19±0.01***	17.54±0.0057***	51.54±0.0028***
IC_{50}	166.43±0.03***	128.10±0.07***	135.57±0.05***	96.06±0.11***	456.93±0.63***	94.80±0.0025***

Values are expressed as mean±SEM (n=3). A: Ascorbic acid, V1: Methanol extract, V2: Chloroform extract, V3: N-butanol extract, V4: Acetone extract and A1: Methanol extract of *Annona reticulate* Linn.

μg/ml show 1.375±0.066, 1.164±0.091, 1.942±0.28, 2.17±0.16, 1.631±0.0721, and 2.17±0.10. *P* value is $P < 0.0001$:***, $P < 0.01$:**, $P < 0.05$:*Fe3+reducing powers assay description is shown in Table 5.

Anti-tubercular activity

Agar cup well method

Extract and fraction sample was tested against three mycobacterium strains, namely, *mycobacterium tuberculosis (M. tuberculosis), mycobacterium Phlei (M. Phlei),* and *Mycobacterium Smegmatis (M. Smegmatis)* by disk diffusion method and well diffusion method. In well diffusion method, *Mycobacterium Smegmatis, Mycobacterium tuberculosis,* and *Mycobacterium Phlei* were inhibited by extract and fractions but activity found was not significant. By the disk diffusion method activity against *M. Tuberculosis* was comparatively good than the well diffusion method. Comparative zone of inhibition agar well diffusion method and disk diffusion method is given in following Tables 6 and 7, respectively. MIC is given in Tables 8 and 9.

Disk diffusion method

Extract and fraction sample was tested against three mycobacterium strains namely *mycobacterium tuberculosis* (*M. tuberculosis*), *mycobacterium Phlei* (*M. phlei*) and *Mycobacterium smegmatis* (*M. smegmatis*) by disc diffusion method and well diffusion method. By the disc diffusion method activity against *M. Tuberculosis* was comparatively good than the well diffusion method. Zone of inhibition of extract and fraction of Borassusflabellifer Linn. andAnnonareticulata Linn.on *M. Smegmatis* (MS) by agar well cup method and disk diffusion method. Shown in Tables 10 and 11.

Table 5: Radical scavenging activity of the extract and fraction of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. by fe^{3+}assay

Concentration in (μg/ml)	V1	V2	V3	V4	A1	A
50	0.291±0.053***	0.625±0.051***	0.432±0.43***	0.637±0.11***	1.229±0.0372***	0.809±0.0057***
100	0.302±0.015***	0.743±0.023***	0.57±0.11***	0.585±0.11***	1.034±0.0196***	0.701±0.0083***
150	0.741±0.079***	0.873±0.063***	0.948±0.076***	0.801±0.50***	1.15±0.1559***	1.401±0.042***
200	0.991±0.097***	1.023±0.040***	1.121±0.077***	1.104±0.96***	1.464±0.0375***	1.846±0.022***
250	1.375±0.066***	1.164±0.091***	1.942±0.28***	2.17±0.16***	1.631±0.0721***	2.17±0.10***

Values are expressed as mean±SEM (*n*=3). A: Ascorbic acid, V1: Methanol extract, V2: Chloroform extract, V3: N-butanol extract, V4: Acetone extract and A1: Methanol extract of *Annona reticulate* Linn.

Table 6: Anti-tubercular activity of extracts and fractions of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. by agar well cup method

Bacterial organisms	Diameter of zone of inhibition mean±SD in mm					
	V1	V2	V3	V4	A1	R
M. Smegmatis.	-	-	7±1.041**	9±0.5774***	8±1.607**	25±0.5774***
M. Tuberculosis	2±0.05774*	2±0.7638*	3±0.500*	12±0.2887***	4±0.5774*	25±0.5774***
M. Phlei	2±0.7638*	2±0.2887*	5±1.155*	10±0.5774***	3±0.2887*	23±0.5774***

Values are expressed as mean±SEM (*n*=3). R: Rifampicin, V1: Methanol extract, V2: Chloroform extract, V3: N-butanol extract, V4: Acetone extract, A1: Methanol extract

Table 7: Anti-tubercular activity of extracts and fractions of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. by disk diffusion method

Bacterial organisms	Diameter of zone of inhibition mean±SD in mm					
	V1	V2	V3	V4	A1	R
M. Smegmatis.	3±0.5774*	5±0.4619*	-	9±0.3464***	2±0.2887*	25±0.5774***
M. Tuberculosis	2±0.7638	-	3±0.5774*	12±0.5774***	-	26±0.2887***
M. Phlei	6±0.2309*	5±0.4619*	5±0.4619*	10±0.2887***	4±0.6928*	23±0.5774***

Values are expressed as mean±SEM (*n*=3). R: Rifampicin, V1: Methanol extract, V2: Chloroform extract, V3: N-butanol extract, V4: Acetone extract, A1: Methanol extract

Table 8: Minimum inhibitory concentration of extract and fraction of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. by agar well cup method

Bacterial organisms	Minimum inhibitory concentration (ug/ml)					
	Agar well cup method					
	V1	V2	V3	V4	A1	R
M. Smegmatis	-	-	-	-	-	-
M. Tuberculosis	5±4041	5±4041	5±0.6351	1.25±0.1443	2.5±02887	0.001
M. Phlei	5±0.5196	5±0.1732	5±0.2887	1.25±0.2887	5±0.1155	0.001

Values are expressed as mean±SEM (*n*=3). R: Rifampicin, V1: Methanol extract, V2: Chloroform extract, V3: N-butanol extract, V4: Acetone extract, A1: Methanol extract

Table 9: Minimum inhibitory concentration of extract and fraction of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. by disk diffusion method

Bacterial organisms	Minimum inhibitory concentration (ug/ml)					
	Disk diffusion method					
	V1	V2	V3	V4	A1	R
M. Smegmatis	-	-	-	-	-	-
M. Tuberculosis	5±0.05774	-	5±0.5196	1.25±0.4330	-	0.001
M. Phlei	1.25±0.2887	2.5±0.2887	2.5±0.5774	1.25±0.4330	5±0.3464	0.001

Values are expressed as mean±SEM (n=3). R: Rifampicin, V1: Methanol extract, V2: Chloroform extract, V3: N-butanol extract, V4: Acetone extract, A1: Methanol extract

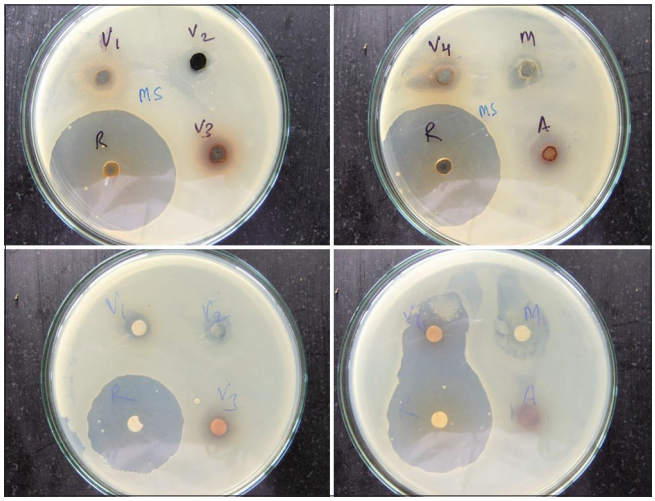

Table 10: Zone of inhibition of extract and fraction of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. on *M. Smegmatis* (MS) by agar well cup method and disk diffusion method. R: Rifampicin, V1: Methanol extract, V2: Chloroform extract, V3: N-butanol extract, V4: Acetone extract, A1: Methanol extract.

Minimum inhibitory concentration (MIC)

Extract and fraction sample was tested against two mycobacterium strains namely *mycobacterium tuberculosis* (*M. tuberculosis*), *mycobacterium Phlei* (*M. phlei*) and by MIC. Detail ivin in Tables 8 and 9.

Discussion

TB remains a leading cause of morbidity and mortality in maximum of the developing world. It is predictable that nearly one-third of the world's population is infected and more than 1.5 million people die of TB every year. Although TB can be treated with a cocktail of antibiotics, current vaccine and chemotherapeutic measures are limited in their efficacy and fail to inhibit the spread of the disease.[32] New anti-tubercular agents are being discovered but it producing resistance.

Plants constitute important source of numerous secondary metabolites. Methanolic extract of *Borassus flabellifer* Linn. illustrates the presence of alkaloid, steroid, triterpenoids, flavonoid, glycosides, and phenolic compound. The present phytochemical having free-radical scavenging activity may be due to phenolic and flavonoids content. Tannins specially inhibit enzymes and substrate deprivation and have ability to scavenge radicals. It also contains spirostane type steroids such as bacosides and dioscin which may have antioxidant and anti-tubercular activity.[33] Methanolic extract of *Annona reticulate* Linn. demonstrates the presence of alkaloid, steroid, triterpenoids,

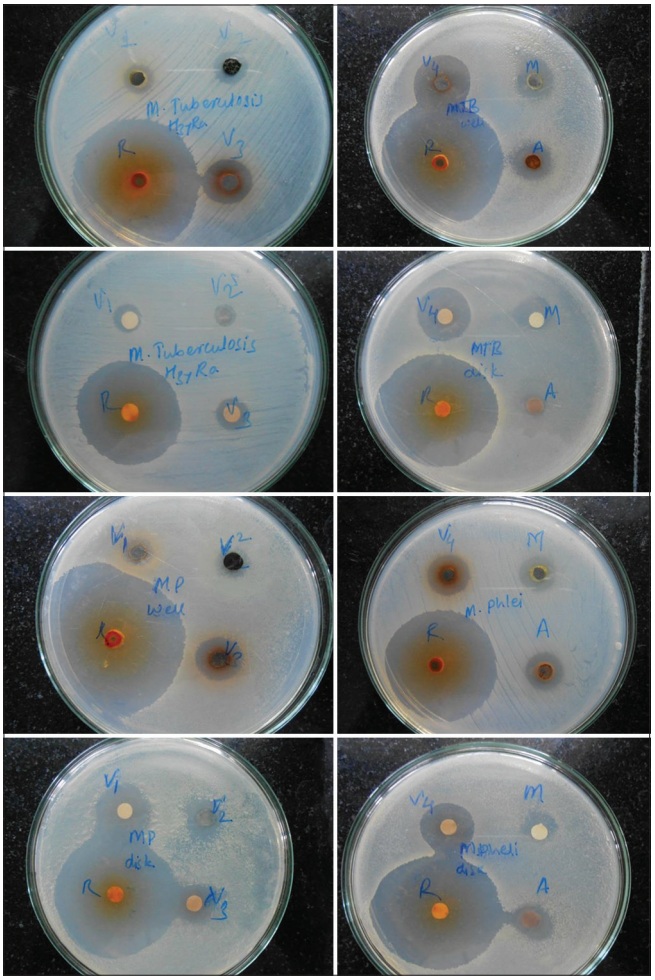

Table 11: Zone of inhibition of extract and fraction of *Borassus flabellifer* Linn. and *Annona reticulate* Linn. on *M. Tuberculosis* (MT) and *M. Phlei* (MP) by agar well cup method and disk diffusion method

flavonoids, glycosides, and phenolic compound. The presence of phytochemicals, phenolic, and polyphenolic constituents might be responsible for the free-radical scavenging activities in the present experiment. The diet rich in antioxidants, polyphenolic compounds may have inhibitory effects on mutagenesis and carcinogenesis in humans that also involved in stabilizing lipid peroxidation. Tannins have antioxidant activity and they have ability to scavenge radicals.[25,34] These entire constituents have same mechanism of action such as antimicrobial and antioxidant.[34]

Reactive oxygen species (ROS) are known to have both deleterious and beneficial effects. Moderate concentration of ROS, normally

generated by the tightly regulated enzymes, provides beneficial physiological effects, for example, in regulation of immune responses, and overproduction results in damage of cell structure, DNA through oxidative stress,[18,35] deplete glutathione, and decrease the activity of antioxidant. Oxidative stress may be a key link between mitochondrial dysfunction and ASD as ROS generated from pro-oxidant environmental toxicants and activated immune cells can result in mitochondrial dysfunction.[18,36] These ROS/RNS are produced during the cellular metabolism play a significant role in cell signaling, apoptosis, gene expression, and ion transportation. However, excessive accumulation of ROS can cause oxidative stress, which results in the damage of DNA, RNA, proteins, and lipids inhibiting their normal functions.[37]

Antioxidant principles present in the plants extract and fractions have been shown to possess free-radical scavenging activity. DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extract and fractions DPPH is a stable, nitrogen-centered free radical which produces violet color in ethanol solution. It was reduced to a yellow colored product, diphenylpicryl hydrazine, with the addition of the fractions in a concentration-dependent manner. The decrease in absorbance of DPPH caused by antioxidants is due to the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation causes discoloration from purple to yellow.[26,38] Similar significant reduction was observed when extract and ascorbic acid treated with DPPH due to their scavenging ability.[25]

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but is an intracellular precursor of hydroxyl radicals which is very toxic to the cell. Thus, scavenging of H_2O_2 is a measure of the antioxidant activity of the plant extract and fractions. All the plant extract and fractions scavenged hydrogen peroxide which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water. Plants extract and fractions have been reported to exhibit antioxidant activity due to the presence of antioxidant compounds such as phenolic and tannins. The plant extract and fractions were shown significant radical scavenging activity.[1,26]

Total antioxidant activities reflect the capacity of a non-enzymatic, antioxidant defense system. In the phosphor molybdenum method, molybdenum VI (Mo6+) is reduced to form a green phosphate/Mo5+ compounds, which have an absorption peak at 695 nm, were generated subsequently. Plant extract and fractions showed a potent total antioxidant activity, which were concentration-dependent.[23,24]

In the reducing power assay, the presence of antioxidants in the sample results in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} can then be monitored by measuring the formation of Perl's blue at 700 nm. Increasing absorbance indicates an increase in reductive ability of extract. The reducing capacity of plant extract and fractions may serve as a significant indicator of its potential antioxidant activity. The activity of antioxidants has been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity, and radical scavenging. Further studies are required to establish the mechanism by which the plant extract and fractions bring about antioxidant activity.[1,26,39]

Anti-tubercular activity of methanolic extract and fractions was evaluated using agar well method. Rifampicin was used as standard for anti-tubercular activity. Acetogenin alkaloids exhibit potent bioactivities through diverse mechanisms such as through depletion of ATP through inhibiting complex I of mitochondria and inhibiting the NADH oxidase of plasma membrane. *A. reticulate* is rich in acetogenin alkaloid the possible biological role these phytochemical as anti-tubercular activity.[40]

The potential anti-tubercular activity of fraction was screened against *M. tuberculosis*, *M. Smegmatis* and *M. Phlei* using agar cup method but methanolic extract not susceptible anti-tubercular activity.

The potential anti-tubercular activity of fraction was screened against *M. tuberculosis*, *M. Smegmatis,* and *M. Phlei* using agar cup method but chloroform fraction not susceptible anti-tubercular activity.

N-butanol fraction potential anti-tubercular activity of fraction was screened against *M. tuberculosis*, *M. Smegmatis,* and *M. Phlei* using agar cup method. From that the fraction has potent anti-tubercular activity against *M. Phlei* as compare to standard drug (rifampicin).

Acetone fraction is effective against *M. tuberculosis*, *M. Smegmatis,* and *M. Phlei* using agar cup method. This fraction shows the highest zone of inhibition against *M. Phlei* as compared to standard (rifampicin). Acetone fraction has antimicrobial potential may be due to the presence of phenols and polyphenols.[41,42]

Methanolic extract is effective against *M. tuberculosis*, *M. Smegmatis,* and *M. Phlei* using agar cup method. This fraction shows the highest zone of inhibition against *M. Phlei* as compared to standard (rifampicin). Methanolic extract shows anti-tubercular activity may be due to the presence of tannin, flavonoid and alkaloids as they are soluble in methanol.[28,42]

Conclusion

In present work conclude that the investigation for extract and fractions of *Borassus flabellifer* Linn. and extract of *Annona reticulate* Linn. shows concentration dependent antioxidant activity. The plant extract and fractions contain probable antimicrobial components that may be used for the development of pharmaceutical industries as a therapy against various diseases. The n-butanol, acetone fraction of *Borassus flabellifer* Linn. and methanol extract *Annona reticulate* Linn. possess significant inhibitory effect against tested pathogens. The results of the study support the development of new anti-tubercular agent drugs from both the plants.

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