Indian J. Applied & Pure Bio. Special Vol. 2(1) 263-271 (2021). **ISSN: 0970-2091**
A web of Science Journal
National e-Conference on Biodiversity Building and Natural Resource Management (NC BBNRM) Date: 22nd-23rd, December, 2021

Evaluation of antimicrobial activity and flavonoid content on some species of *Zingiberaceae* family

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Abstract

The present study deal with the biological activity and flavonoid content of the dried rhizomes of *Alpinia nigra, Alpinia galanga, Amomum gracile, Curcuma leucorhiza, Costus speciosus, Curcuma amada, Hedychium rubrum, Hedychium maximum, Kaempferia rotunda* and *Hedychium coronarium* belonging to the family of Zingiberaceae. Methanol was used to extract the active bio-compounds from the plants that were pre dried and the crude extract was further used for the antimicrobial study and also for determining the flavonoid content. The values are presented as mg quercetine equivalent per gram of the sample's extract and the values are arrange as: *Curcuma leucorrhiza* (312.61), *Alpinia nigra* (199.57), *Curcuma amada* (197.43), *Hedychium maximum* (183.33), *Hedychium coronarium* (138.46), *Hedychium rubrum* (129.06), *Amomum gracile* (97.43), *Alpinia galanga* (92.31), *Costus speciosus* (68.80) and *Kaempferia rotunda* (22.65) respectively. For antibacterial activity, we have used three bacteria namely, *Micrococcus luteus*, *Bacilluus subtilis* and *Escherichia coli*; Chloramphenicol is used as the standard microbics and the results are presented in mm (zone of inhibition).

Medicinal plants have become more important for primary health care system throughout the world especially in developing countries. For the development of novel therapeutic agents for the treatment of human diseases, many pharmaceutical companies have carried out investigation to identify new drugs or to find new lead structures[17]. Since plants chemical have the properties for prevention and treatment of various human diseases. It is reported that a wide variety of plant compound used in ethano-medicine has antimicrobial activity against various microorganisms.[15] Due to their wide pharmacological activity, use of medicinal plants as traditional medicine is one of the common practice in

India. Since time immemorial, many herbal practitioners such as Ayurveda, Unani and Siddha have been practicing the use of herbal medicines on the basis of treatment and cure for various diseases in physiological conditions[11]. In the last four decades, pharmacological companies have produced a number of new antibiotics but because of the continuous use of the antibiotic, microorganism became resistant against such antibiotics. As the resistance of microbes develops, uncertainty in the use of antimicrobial drugs in the future still remains. Therefore, development of new substitutes for antimicrobial drugs is required for the treatment of various infectious diseases; an alternative way to come up with this issue is to have a scientific approach towards local medicinal plants for such possible properties[13].

Plants and its materials has always been a vital source to deal with serious diseases in the world. According to World Health Organisation, 80 % of the total population of the word depends on traditional medicine which is largely plant based, for their primary healthcare needs. In recent time, plant has become the potent antimicrobial agent. Traditionally used medicinal plant was once regarded as the sole source of treatment, making it a focus in the search for solution to increasing drug resistance among pathogenic microorganisms. In many countries, traditional medicine has been long accepted as an alternative to western medicinal practice[14]. Many researchers have reported that many plant species belonging to Zingiberaceae family have potent antimicrobial activity[1 – 6, 8 – 10, 15].

Plant collection and identification: Fresh and healthy rhizomes of ten plant species of Zingiberaceae family (*Alpinia nigra, Alpinia galanga, Amomum gracile, Curcuma leucorhiza, Costus speciosus, Curcuma amada, Hedychium rubrum, Hedychium maximum, Kaempferia rotunda* and *Hedychium coronarium*) was collected during the month of January - May from Kangmong area, Imphal, west district, Manipur, India. The rhizomes were washed with running water for removing adhered particles. The plant samples that were collected were identified by Prof. P. Kumar Singh, Advance Study Centre Life Science Department, Manipur University, Canchipur, Imphal, Manipur, India.

Extract preparation :

The clean rhizomes were cut into thin slice; shade dried for about seven days, it was then converted into fine powder with the help of a mechanical grinder and then transferred into a clean air tight container for further studies. 50mg of the plant powder samples were soak in 500ml of methanol (Hi-Media) with occasional shaking for three days. The solvent was filtered for the total extract using Watt-man no. 1 filter paper, evaporate the filtrate, collect the crude mass into a clean glass container and kept for further use.

Antimicrobial activity :

Two Gram positive (*Micrococcus luteus* and *Bacillus subtilis*) and one Gram negative bacteria (*Escherichia coli*) were used for this study, these three organism were collected from Biochemistry Department, Manipur University, Canchipur, Imphal, Manipur, India.

Preparation of medium :

14.8gm of nutrient agar and 3.2gm of agar powder were added to 400ml of distilled water in a 500ml conical flask. The suspension was heated for about 4 min. in a hot plate to dissolve the nutrient agar and agar powder. After complete dissolution of the media, the mouth of the conical flask was closed tightly by aluminium foil. The media was then autoclaved at 121°C for 15 min.

Preparation of agar plates :

Petri plates were sterilized in a hot air oven at 160°C for 2 hours. The plates were then allowed to cool. 25ml of the hot media was then poured into each sterilized plates and the medium was allowed to form into gel. The agar plates were then covered with aluminium foil and transferred in refrigerator until use.

Preparation of stock solution of extracts:

Stock solution of the extracts were prepared by dissolving 20mg of each of the crude extracts in 1ml dimethyl sulphoxide (DMSO) to give a concentration of 20mg/ml.

Preparation of saline solution :

100ml of the saline solution was prepared by dissolving 0.9gm sodium chloride in 100ml distilled water and was sterilized before preparation of microbial suspensions, at 121°C for 15min. in an autoclave.

Preparation of bacterial suspension :

Bacterial broth cultures of the respective microorganisms were prepared to a density of 10^8 cells ml^{-1} of 0.5 McFarland standards.

Antimicrobial activity :

The antimicrobial assay was performed by agar disc diffusion method[16]. The petri plates containing the media were then inoculated with 100µl of 0.5 concentration of the test organism and left for 30 min. to dry. The sterile disc (0.7cm) (Hi-media) was saturated with 25µl of the test compound and was introduced on the upper layer of the seeded agar plates. The plates were incubated overnight at 37°C. The growth of the microbes was determined by measuring the diameter of inhibition zone (in mm). As a negative control, DMSO was used. The experiment was done in triplicate and the final mean values were recorded. The results were compared with the standard antimicrobics Chloramphenicol (25µg/ disc).

Estimation of flavonoid [7]:

10gm of Quercetin was dissolved in 5ml of ethanol making 2mg/ml as stock solution. 0.05to 0.25ml of aliquots from the stock solution were pipette out into 25ml volumetric flask. The volumes of all the contents were made to 0.5ml by adding ethanol. At the same time the plant extract and 0.1ml of 20mg/ml DMSO solution were separately mixed with 0.4ml of ethanol making volume to 0.5ml. 0.3ml $NaNO_2$ solution was added to both standard quercetin and plant samples, voltexed well and kept for 5 minutes. 0.3ml of $AlCl_3$, 2ml of 1M NaOH and 1.9ml of distilled water were added to both the samples and the standard Quercetin solutions. A blank sample of 0.5ml of ethanol with the above reagent was set up; absorbance

at 510nm wavelength was recorded against the blank sample. A calibration curve for standard Quercetin was plotted as absorbance vs concentration. From this graph the amount of flavonoid content was determined as quercetin equivalent.

Flavonoids are plant phytochemical with a variable phenolic structure, it possess many biological activity such as antioxidant, antimicrobial, hepatoprotective, anti-inflametory, anticancer and antiviral activity. In our study flavonoid content is presented as milligram quercetine equivalent (QE) per gram of the sample extract. Here Fig. - 1 and Fig. - 2 show the flavonoid content in the plant samples and calibration curve of standard quercetine. From the result of calculation, flavonoid content (Table-1) was found highest in *Curcuma leucorhiza* (312.61), lowest value is found in *Kaempferia rotunda* (22.65) and the values are arrange in the flowing decresing order as: *Curcuma leucorhiza* (312.61) > *Alpinia nigra* (199.57) > *Curcuma amada* (197.43) >*Hedychium maximum* (183.33)> *Hedychium coronarium* (138.46) > *Hedychium rubrum* (129.06) > *Amomum gracile* (97.43) > *Alpinia galanga* (92.31) > *Costus speciosus* (68.80) > *Kaempferia rotunda* (22.65).

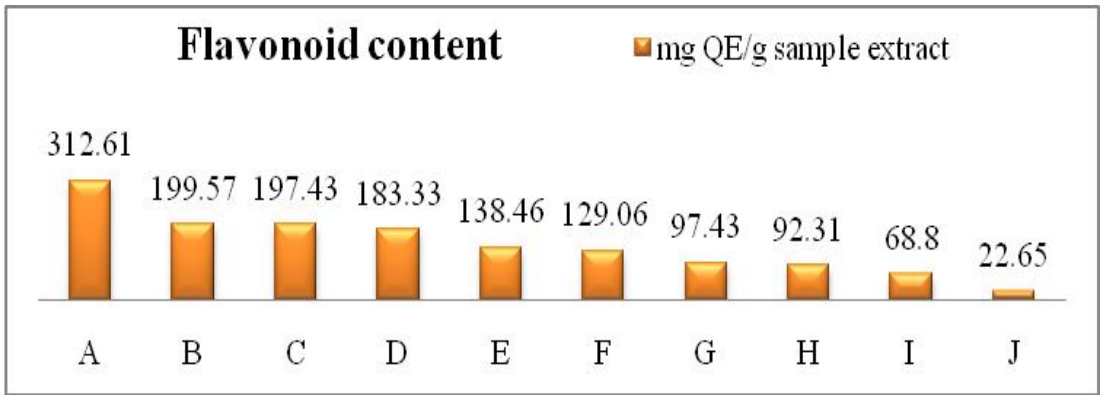
Antimicrobial analysis :

In-vitro antimicrobial studies of ten selected medicinal plants (rhizome parts only) was evaluated against three bacterial species (*Escherichia coli*, *Bacillus subtilis* and *Micrococcus luteus)* by the presence or absence of inhibition zone, diameter of inhibition zone. In general, the ten plant species possesses varying degree of antimicrobial activity against the tested microorganism (Table- 2).

Table-2 illustrated that all the plant rhizome of *Alpinia nigra, Curcuma amada, Hedychium maximum, Hedychium coronarium, Hedychium rubrum, Amomum gracile, Alpinia galanga, Alpinia galanga, Costus speciosus* and *Kaempferia rotunda* except

Table-1. Flavonoid content in methanol extract expressed in mg QE/ gm of the sample extract

Sample name	mg QE/g sample extract	Rank
Curcuma leucorhiza	312.61	1
Alpinia nigra	199.57	2
Curcuma amada	197.43	3
Hedychium maximum	183.33	4
Hedychium coronarium	138.46	5
Hedychium rubrum	129.06	6
Amomum gracile	97.43	7
Alpinia galanga	92.31	8
Costus speciosus	68.80	9
Kaempferia rotunda	22.65	10



Note: A = *Curcuma leucorhiza,* B = *Alpinia nigra,* C = *Curcuma amada,* D = *Hedychium maximum,* E = *Hedychium coronarium,* F = *Hedychium rubrum,* G = *Amomum gracile* ,H. = *Alpinia galanga,* I = *Costus speciosus,* J = *Kaempferia rotunda*

Fig.-1 Graph showing the Flavonoid content in methanolic extract of the samples expressed in mg QE/gm of the plant extract

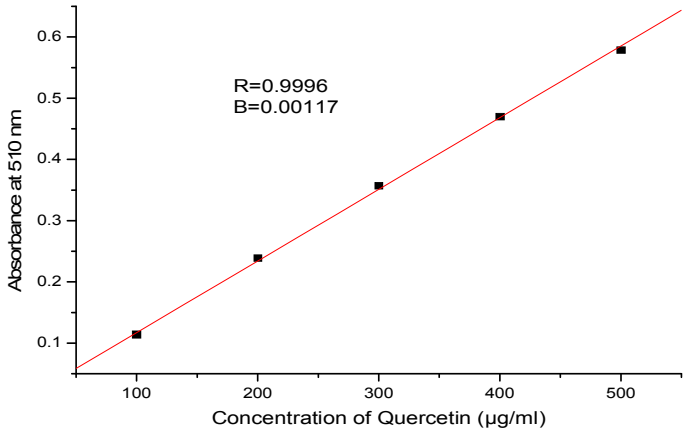


Fig.-2 Calibration curve of Quercetin

Curcuma leucorhiza give positive test against all the test organisms, except for *Curcuma leucorhiza* which does not show positive test against *Micrococcus luteus* and *Escherichia coli*. It was also observed that *Hedychium maximum* and *Kaempferia rotunda* exhibited the highest and lowest activity (17.5 and 10mm) respectively against *Bacillus subtilis*. In case of *Micrococcus luteus*, highest value of activity of the plant sample were observed in *Hedychium maximum* (19mm) and lowest activity was observed both in *Kaempferia rotunda* and *Costus speciosus* (10mm). *Hedychium coronarium* observed the highest antibacterial activity (16mm) against *Escherichia coli* while the plant samples observed minimum value.

Table-2 Inhibition zone of plant samples against the test microorganisms

Sample name	Concentration	Zones of inhibition (mm)		
		Bacillus subtilis	*Micrococcus luteus*	*E. coli*
Hedychium maximum	20mg/ml	17.5	19	11.5
Hedychium rubrum	20 mg/ml	11.5	10.5	10
Hedychium coronarium	20 mg/ml	16.7	15	16
Kaempferia rotunda	20 mg/ml	10	10	11.5
Amomum gracile	20 mg/ml	12.5	14	10
Alpinia galanga	20 mg/ml	11.5	11	10
Curcuma amada	20 mg/ml	11.3	12	10
Alpinia nigra	20 mg/ml	17	12	13
Curcuma leucorhiza	20 mg/ml	11	-	-
Costus speciosus	20 mg/ml	15	10	10
Chloramphenicol	25µg/disc	20	19	24

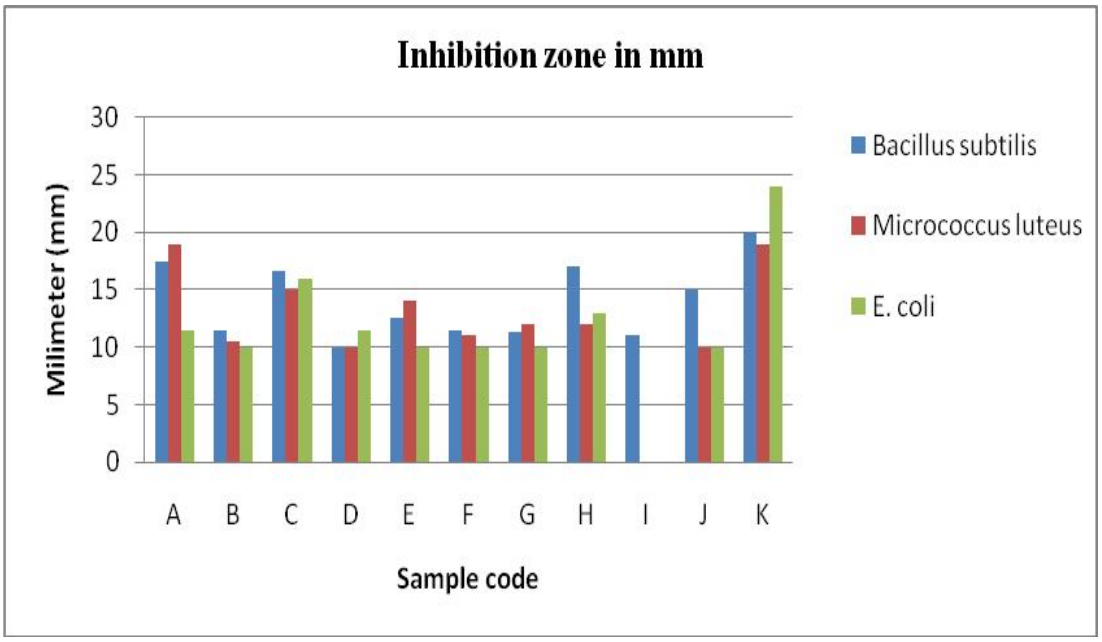
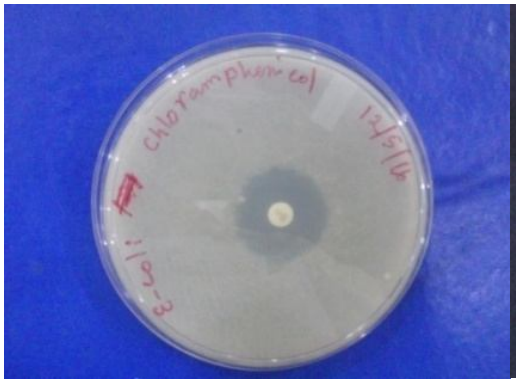


Fig. 3 Graph showing inhibition zone in mm of the plant samples against the tested bacteria
Note: A = *Hedychium maximum*, B = *Hedychium rubrum*, C = *Hedychium coronarium,* D = *Kaempferia rotunda*, E = *Amomum gracile*, F = *Alpinia galanga*, G = *Curcuma amada*, H = *Alpinia nigra*, I = *Curcuma leucorhiz*a, J = *Costus speciosus*, K = Chloramphenicol

Photograph showing inhibition zone



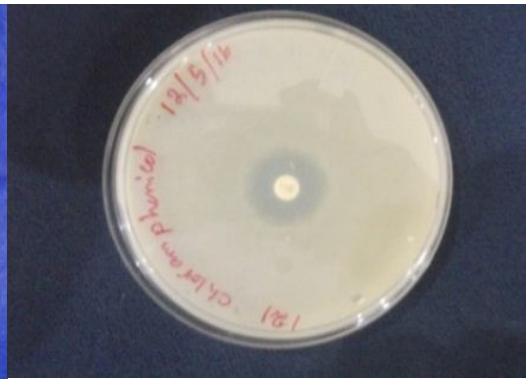
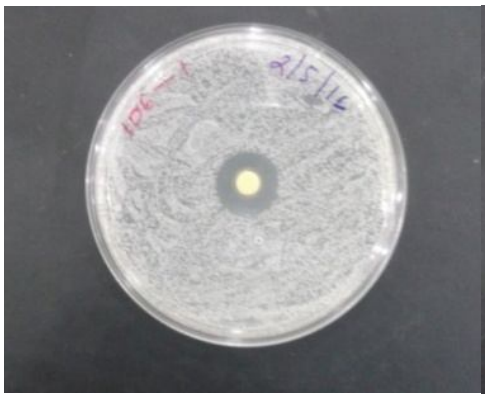


Fig- 4 Fig- 5

Fig- 4 & 5 *Standard drugs* against *E. coli* & *Bacillus subtilis*



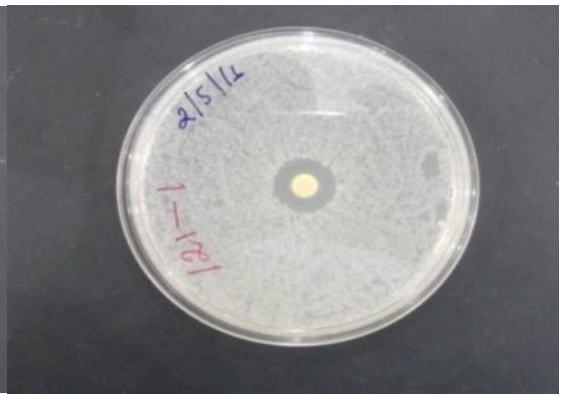


Fig-6 Fig.-7

Fig- 6 & 7 *Hedychium maximum* against *Micrococcus luteus* & *Bacillus subtilis*

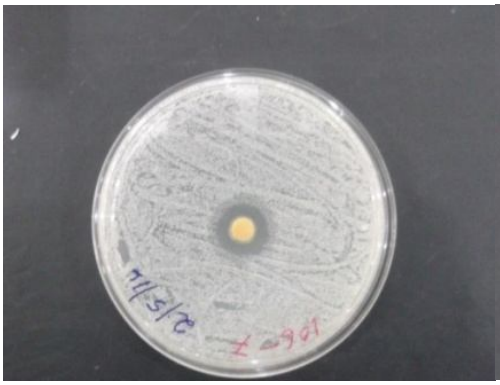


Fig-8 Fig. 9

Fig- 8 & 9 *Curcuma amada* against *Micrococcus luteus* & *Bacillus subtilis*.

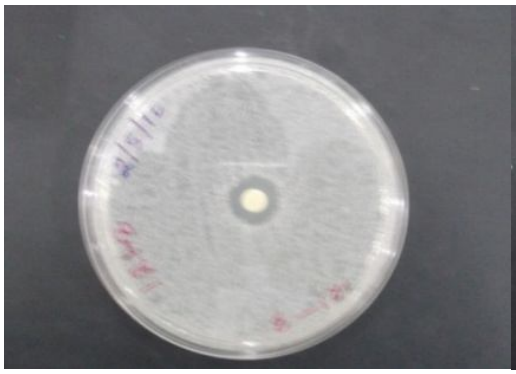


Fig.10

Fig. 11

Fig- 10 & 11 *Hedychium coronarium* against *Bacillus subtilis & Micrococcus luteus*

The present study was taken up due to the rising resistance of microbes against certain drugs.

Since extracts of plants and its compounds are gaining much interest not only in wellness purposes but also as good antimicrobial agents, a total of ten plants were selected to check their antimicrobial property from its rhizome extract against some common pathogens. In general, *Hedychium maximum* rhizome extracts appeared to be an effective source of active antimicrobial agents against *Bacillus subtilis* and *Micrococcus luteus* (20mm and 19mm). However, *Hedychium coronarium* (16mm) recorded to possess higher antimicrobial activity among the other tested medicinal plants. From our findings as well as from literature survey we may come to the conclusion that the plants selected for this study possesses good to average potential to combat such microbial issues. On the other hand, further investigations and studies needs to be carried out to avail maximum benefit from such medicinal plants, not only as antimicrobial agents but also in other therapeutic properties. Thus, we can come to the conclusion that the selected plants for this study can be used as an anti-microbial agent against various microbes/pathogens.

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