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# ISOLATION AND PHYTOCHEMICAL INVESTIGACTION IN THE LEAVES OF *DURIO ZIBENTHINUS* LINN FOR ANTIBACTERIAL ACTIVITY

**V S Thiruvengadarajan*, A. Rajasekeran, I. Ponnilavarasan, M. Sangeetha, R. Arivukkarasu**

Department of Pharmaceutical Analysis, KMCH College of Pharmacy, Coimbatore, TN

***Corresponding Author E-mail:** vstrajan1679@gmail.com

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**ABSTRACT**

In ancient period, people mainly depended on plant and animal sources for their survival. Natural products are natural substances that have their own pharmacological and biological activity. Group of about 4000naturally available polyphenolic compounds are flavonoid, presence universally in plant sources. Durian is an occasional natural product that generally well known in Southeast Asia, especially Malaysia, Indonesia, Thailand, and Philippines. Ethnobotanical importance of durian also influenced this research and is fast becoming a main strategy for drug development. The aim of the present research work is to isolate the lead compound and study the anti-microbial activity of isolated compound. Flavonoids have various pharmacological activity due its specific chemical structure which include some flavonoids have antioxidant activity by scavenging free radicals in organisms. Flavonoids also have some other pharmacological activity like anti tumor, anti-inflammatory, antibiosis, antiviral activity, inhibiting the activity of enzymes and so on. Durian is an occasional natural product that generally well known in Southeast Asia, especially Malaysia, Indonesia, Thailand, and Philippines. The aim of this research work is to isolate and study the phytoconstituents for antibacterial activity. The determination of antibacterial activity helps in establishing these compounds as medicine for further drug development. The flavonoid compound was successfully isolated from the ethyl acetate extract of the leaves of Durio Zibenthinus. And the isolated

## INTRODUCTION:

In ancient period, people mainly depended on plant and animal sources for their survival. Natural products are natural substances that have their own pharmacological and biological activity. Medicinal plants are gaining accepted in large now a days probably due to insufficiency of the modern drugs to cure many diseased such as syphilis, typhoid, TB and various bacterial infections due to increase in bacterial resistance and increase in the cost of the antibiotics. Now a days with the advancement of automated high Throughput experimental methods, pharmaceutical industry renewed commitment to searching for new medicines from plant origin[1]. Group of about 4000naturally available polyphenolic compounds are flavonoid, presence universally in plant sources. Flavonoids are classified based on variation in their functional group and their position of the 15-C skeleton like flavone, flavanone, flavonol, isoflavonoid, anthocyanidin and chalcones[2]. Flavonoids have various pharmacological activity due its specific chemical structure which include some flavonoids have antioxidant activity by cavenging free radicals in organisms[3]. Flavonoids also have some other pharmacological activity like

anti tumor, anti-inflammatory, antibiosis, antiviral activity, inhibiting the activity of enzymes and so on[4-10]. Additionally flavonoids act as weak hormones at treating women's menopausal syndrome[11-14]. Durian is an occasional natural product that generally well known in Southeast Asia, especially Malaysia, Indonesia, Thailand, and Philippines[15]. Commonly known as civet fruit in English, Durian in Spanish, Dulian in Indonesia, Thurian in Thailand and Mulnari in Tamil[16]. As per research, durian seed can be utilized as a thickener due to the substance of hydrocolloid (water-soluble gum)[17]. Durian have been reported for various pharmacological effects and also screened for phytochemicals. Ethnobotanical importance of durian also influenced this research and is fast becoming a main strategy for drug development. The aim of the present research work is to isolate the lead compound and study the anti-microbial activity of isolated compound. The objectives of the study are

- To identify the presence of lead compound from the leaves of *Durio zibenthinus*. L. plant by High Performance Thin Layer Chromatography(HPTLC).
- To isolate the lead compound by Extrusion method.
- To perform Anti-microbial screening of isolate by diffusion method and broth dilution method (MIC).

**PLANT PROFILE[18-21]:**

***Durio Zibenthinus***.

Botanical name: Durio Zibenthinus.

Synonym : Durio acuminatissima, King of fruits.

**Taxonomical classification**

Kingdom -Plantae

Divison –Magnoliophyta

Class –Magnoliopsida
Order – Malvales
Family –Bombacaceae
Genus – Durio
Species – Zibenthinus

**Ethnobotanical uses[22]:**

Generally, in Asia, the durian leaf and root decoctions have accepted to show antipyretic effect and decoctions are utilized as a febrifuge and hostile to malarial specialist. It is additionally used to treat mucus, diminish colds, and treat skin sicknesses, jaundice, and swellings. The durian organic product is accepted to have warming properties on the body. Durian natural product is considered to have potential medicinal and remedial properties that incorporate its capacity to support the insusceptible framework and wound healing.

**Fig.1: Dorsal surface of durian leaves**

**Fig.2: Ventral surface of durian leaves**

**MATERIALS AND METHODS**

**Collection and Authentication:**

Fresh durian leaves were collected from Anthony nursery, Burliar, Nilgiris district which is on the way from Metupalayam to ooty. The plant was authenticated at Botanical survey of India, Coimbatore, Tamil Nadu.

**Preparation of various extracts:**

The leaf samples were cleaned under running tap water and made free from sand and impurities, properly shadow dried. The materials are coarsely powdered and the powder was passed through the sieve no.60 then stored in a tightly closed container. First 250 gm ofdurian powder was defatted using petroleum ether, then various extracts were prepared using Chloroform, Ethyl acetate, Methanol and Water by cold

maceration extraction process in the ration of 1:3. The so obtained extracts were concentrated in the water bath temperature not exceeding 45ºC and the residue was collected. The residue was then dissolved in methanol and used for further analysis.

**Qualitative phytochemical analysis of various extracts of Durian[23-25]:**

The above obtained extracts of the *Durio zibenthinus* leaf were subjected to identification of active constituents by performing qualitative tests.

**Identification of compound in ethyl acetate extract by HPTLC:**

From the result of preliminary test, ethyl acetate extracts contains flavonoids compound, it can be further identified using High Performance Thin Layer Chromatography. The HPTLC analysis was performed on CAMAG Linomat 5 instrument with stationary phase using silica gel with mesh size passing 350 (SDFCL Mumbai) as adsorbent, self indicator Aluminium TLC plates 10X10cm. TOLUENE:ETHYL ACETATE:FORMIC ACID:METHANOL in the ratio of 3:6:1.6:0.4 were used as mobile phase. Quercetin was placed as a flavonoidal standard. The plate was developed in Twin Trough Chamber 10x10cm. The mobile phase was allowed to migrate up to 8 cm from the bottom of the plate. Then the plate is dried and visualized under UV chamber at 254 and 366nm.

**Isolation of compound by extrusion method[26-27]:**

TLC plate (20 X 20 cm) for separation was manually prepared with silica gel and was activated at 110ºC for 30 minutes. The mobile phase used was Ethyl acteate: Chloroform (50:50) for the proper separation of the required band. The sample was spotted and developed in the twin trough chamber containing 70ml of solvent system. After development the plate was air dried and examined under UV chamber at 366 nm. The developed plate was then scale marked from 0 to 20 cm and placed under UV chamber t 366 nm. The bands were then marked on the scale. The marked area was then scrapped along with silica with spatula and collected in Erylenmayer flask. The same solvent system Ethyl acetate: Chloroform (50:50) was used for the extraction of constituent from the silica. The content was then concentrated by evaporating the solvent at room temperature and the content was marked as Compound DC- 1 and stored.

**Confirmation of isolated compound:**

To know the purity of isolated compound and confirmation purpose, High performance thin layer chromatography was performed using flavonoid Quercetin as standard and compared with HPTLC of extract. The HPTLC analysis was performed on CAMAG Linomat 5 instrument with stationary phase using silica gel as adsorbent, self indicator Aluminium TLC plates 10X10cm. TOLUENE: ETHYL ACETATE: FORMIC ACID: METHANOL in the ratio of 3:6:1.6:0.4 were used as mobile phase. Quercetin was placed as a flavonoidal standard. The plate was developed in Twin Trough Chamber 10x10cm. The mobile phase was allowed to migrate up to 8 cm from the bottom of the plate. Then the plate is dried and visualized under UV chamber at 254 and 366nm.

**Anti-bacterial screening of the isolated compound by disk diffusion method[28-29]**

**Preparation of Nutrient agar medium:**

Appropriate quantity of nutrient agar medium was taken and dissolved in 200 ml of distilled water in a conical flask. Heat the medium if necessary to dissolve it completely. The prepared media was sterilized in autoclave for 15 minutes at 15 lb pressure and 121ºC.

**Preparation of plate:**

Petri plates are sterilized in autoclave for 15 minutes at 15 lb pressure and 121ºC. Sterilized medium was carefully transferred into sterile petri plates aseptically. The petri plates were then allowed to solidified at room temperature. The layers of the medium should be in uniform thickness (thickness of 5-6mm) is done by placing the petri plates on a leveled surface. Gram positive standardized inoculums of *Bacillus subtilis, Staphylococcus albus, Bacillus lintus, Micrococcus luteus, Streptococcus aureus* and gram negative standardized inoculums

of *Escherichia coli, Pseudomonas aeruginosa, Salmonella Paratyphi, Vibrio cholera, Klebsiella pneumonia* were innoculated on the surface of the plate using non-absorbent sterile cotton swab. Sterile disc impregnated with isolated solution (DZ-1 10µg/disc) and standard ciprofloxacin (10µg/disc) were placed on the inoculated agar medium using sterilized forceps. All the plates were placed for incubation at 37ºC for 24 hours. After incubation time, the zone of incubation of sample and standard was measured in mm.

**Determination of Minimum Inhibitory Concentration (MIC) of isolate by broth dilution method:**

- The standardized MIC tubes were labeled 1 to 8 and 1 ml of nutrient agar broth solution was added to each of the tubes.
- The MIC tubes were sterilized in autoclave at 15 lb pressure, 121ºc for 15 minutes and transferred to aseptic room.
- One ml of isolate solution (Compound DZ-1) was aseptically added to the first tube, mixed well and 1 ml was serially transfered up to tube 7.
- The eighth tube acts as control.
- A loop full of standardized test organisms were inoculated to all tubes including control mixed and incubated for 24 hours at 37ºC.
- The highest dilution of isolate showing no turbidity was recorded, and this concentration was believed to contain isolate that required minimum to control the growth of respective test organism.

## RESULT AND DISCUSSION

**Extraction:**

The leaf powder of Durio Zibenthinus (250 gms) were extracted successively with chloroform, ethyl acetate and methanol by cold maceration extraction process and concentrated in a water bath and stored at 4º C in a refridgerator for further analysis. The yield of various extracts were Petroleum ether 2.186g, Ethyl acetate 3.461 g, Chloroform 2.597 g, Methanol 1.764 g, Water 2.178 g.

**Identification of compound in ethyl acetate extract by HPTLC:**

From the result of preliminary test, ethyl acetate extracts contains flavonoids compound, it can be further identified using High Performance Thin Layer Chromatography. The Rf value of standard quercetin matches with the Rf value of eluent in the ethyl acetate extract. That band containing compound was appeared as bright blue fluorescent under UV chamber at 366 nm and then it was subjected for isolation. The Rf values are given in a table 2.

**Qualitative phytochemical analysis of various extracts of Durian:**

| Name of the test | Pet.ether extract | Chloroform extract | Eth.acetate extract | Methanol extract | Water |
|---|---|---|---|---|---|
| Test for Alkaloids (Dragendroff's test) | - | - | - | - | - |
| Test for carbohydrate (Fehling's test) | - | - | - | + | + |
| Test for proteins (Ninhydrin test) | - | - | + | + | - |
| Test for glycosides (Borntrager's test) | - | - | - | + | + |
| Test for flavonoids (Shinoda test) | - | - | + | - | - |
| Test for tannins | - | - | - | + | + |
| Test for steroids (Libermannburchard's test) | + | + | + | - | - |
| Test for fixed oils | + | + | - | - | - |
| Test for saponins (Foam test) | - | + | - | - | - |

**Table.1: Phytochemical analysis of various extracts**

| Peak | Rf values | Standard Rf value (Quercetin) |
|---|---|---|
| 1 | 0.06 | 0.80 |
| 2 | 0.09 | |
| 3 | 0.17 | |
| 4 | 0.20 | |
| 5 | 0.33 | |
| 6 | 0.47 | |
| 7 | 0.53 | |
| 8 | 067 | |
| 9 | 0.74 | |
| 10 | 0.81 | |
| 11 | 0.84 | |

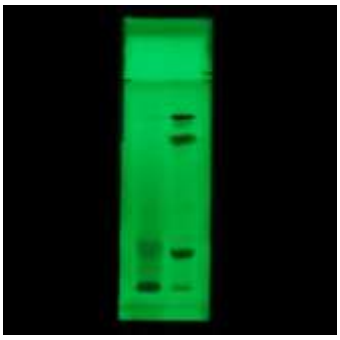

**Fig.3: At 254nm under UV chamber**

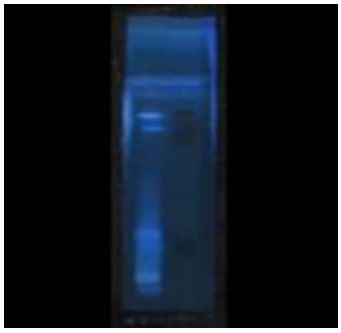

**Fig.4: At 366 nm under UV chamber**

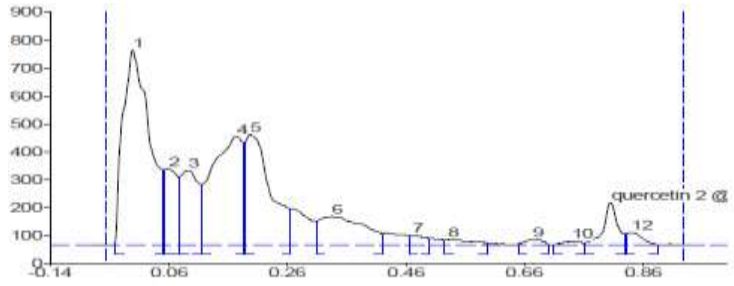


**Fig.5: Chromatogram of ethyl acetate extract.**

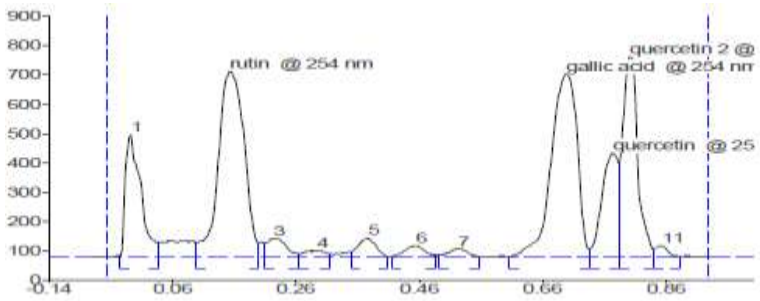


**Fig.6: Chromatogram of standard**

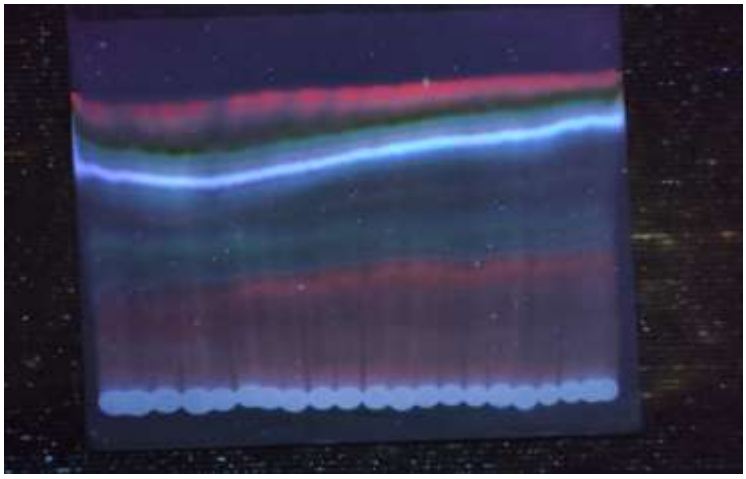

**Fig.7: Preparative TLC under 366 nm.**

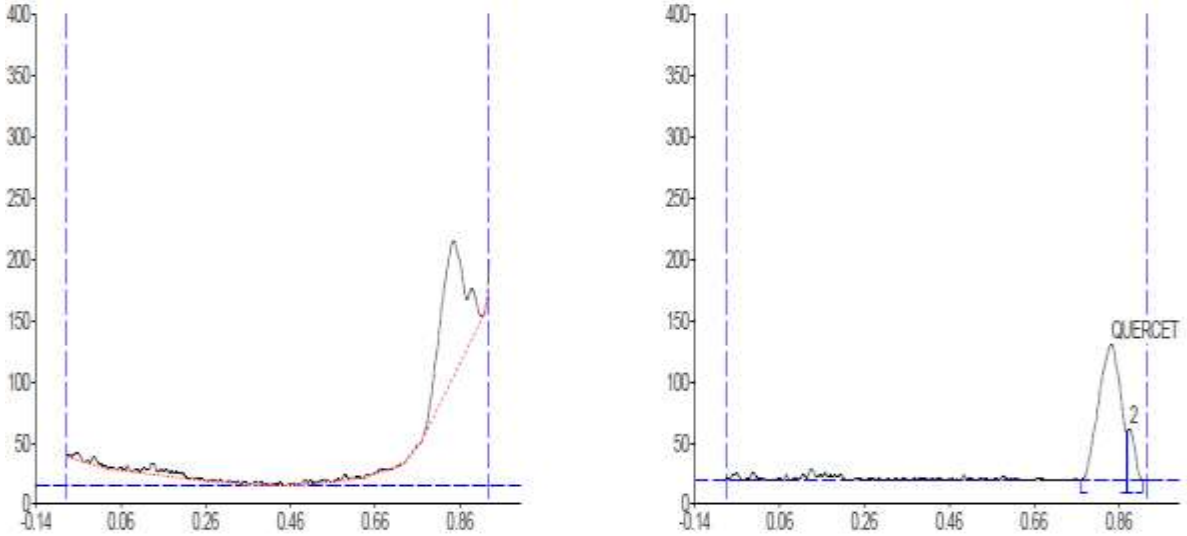


**Fig.8: Chromatogram of isolated compound (DC-1) and standard (Quercetin**

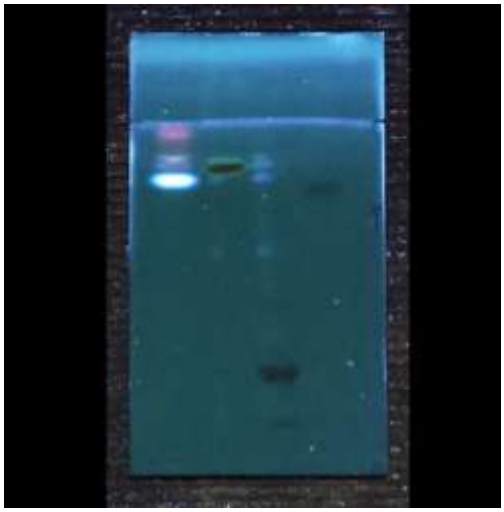

**Fig.9: HPTLC plate of isolated compound (DC-1) at 366 nm.**

***In vitro* Anti-bacterial screening of the isolated compound DC-1:**

| Zone of inhibition | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| COMPOUNS | MICROORGANISMS | | | | | | | | | |
| | *B.subtils* | *S. albus* | *B.lintus* | *M.luteus* | *S. aureus* | *E. coli* | *P.aeruginosa* | *S.Paratypi* | *V.cholera* | *K.pneumonia* |
| Compound (Dc-1) | 15 | 7 | 8 | 0 | 8 | 0 | 0 | 0 | 9 | 0 |
| Standard (Ciprofloxacin | 37 | 30 | 40 | 30 | 30 | 37 | 35 | 30 | 32 | 33 |

**Table.3: In vitro Anti-bacterial screening of the isolated compound DC-1**

**Fig.10: ZOI of Bacillus subtilis**

**Fig.11: ZOI of Staphylococcus albus**

**Fig.12: ZOI of Bacillus lintus**

**Fig.13: ZOI of Klebsiella pneumonia**

**Fig.14: ZOI of Vibrio cholera**

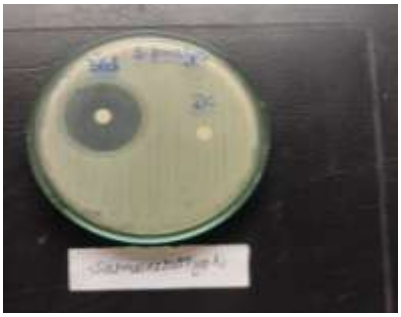

**Fig.15: ZOI of Salmonella Typhi**

| Minimum Inhibitory Concentration (MIC) of isolate | | | | | |
|---|---|---|---|---|---|
| **COMPOUNDS** | **MICROORGANISMS** | | | | |
| | *Bacillus subtilis* | *Staphylococcus albus* | *Bacillus lintus* | *Streptococcus aureus* | *Vibrio cholera* |
| Compund (DC-1) (mg/ml) | 12.5 | 12.5 | 6.25 | 12.5 | 0.78 |

**Fig.16: ZOI of Pseudomonas aeruginosa**

**Fig.17: ZOI of Escherichia coli**

**Fig.18: ZOI of Micrococcus Luteus**

**Fig.19: ZOI of Streptococcus aureus**

**Isolation of compound by extrusion method:**

For isolation of lead compound from the ethyl acetate extract of durian, the qualitative correlation of thin layer techniques with extrusion column chromatography has been applied effectively. Mobile phase was selected by trial and error method. The mobile phase selected for isolation of lead compound was Chloroform: Ethyl acetate (Ratio 1:1). The developed TLC plate showed four bands with Rf values 0.31 (Dark reddish brown in colour), 0.57 (Bright blue fluorescent in colour), 0.7 (Light green in colour), 0.76 (Dark green in colour). Bright blue fluorescent colour band was selected for isolation. That band was scrapped with the help of spatula & extracted using the same mobile phase and concentrated. The isolated compound was named as DC-1.

**Confirmation of isolated compound:**

The purity of isolated solution was confirmed by HPTLC analysis and Rf value of isolated compound was compared with Rf value of standard quercetin. Then the purity of isolated solution was confirmed by scanning in the UV-Vis spectrophotometer in the range of 200-800 nm which produce single peak. The $\lambda_{max}$ was found to be 281.0 nm. Rf value of compound DC-1 was 0.84 and Rf value of standard quercetin was 0.84.

**CONCLUSION:**

From the above research work, it is clear that *Durio zibenthinus* leaves contains flavonoids compound. The flavonoid compound was successfully isolated from the ethyl acetate extract of the leaves of *Durio Zibenthinus.* And the isolated compound showed significant antibacterial activity against tested microorganisms. The overall results provide scientific evidence for isolation, anti-bacterial screening of leaves from the *Durio zibenthinus* Linn plant.

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