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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACT OF *LORANTHUS PARASITICUS* MERR. WITH REFERENCE TO THEIR ANTIBACTERIAL ACTIVITY

Souradut Ray, *Amal Kumar Mondal and Nagendra Kumar Verma

*UGC-DRS-SAP Department of Botany and Forestry, Vidyasagar University, Midnapore- 721102, West Bengal, India

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ABSTRACT

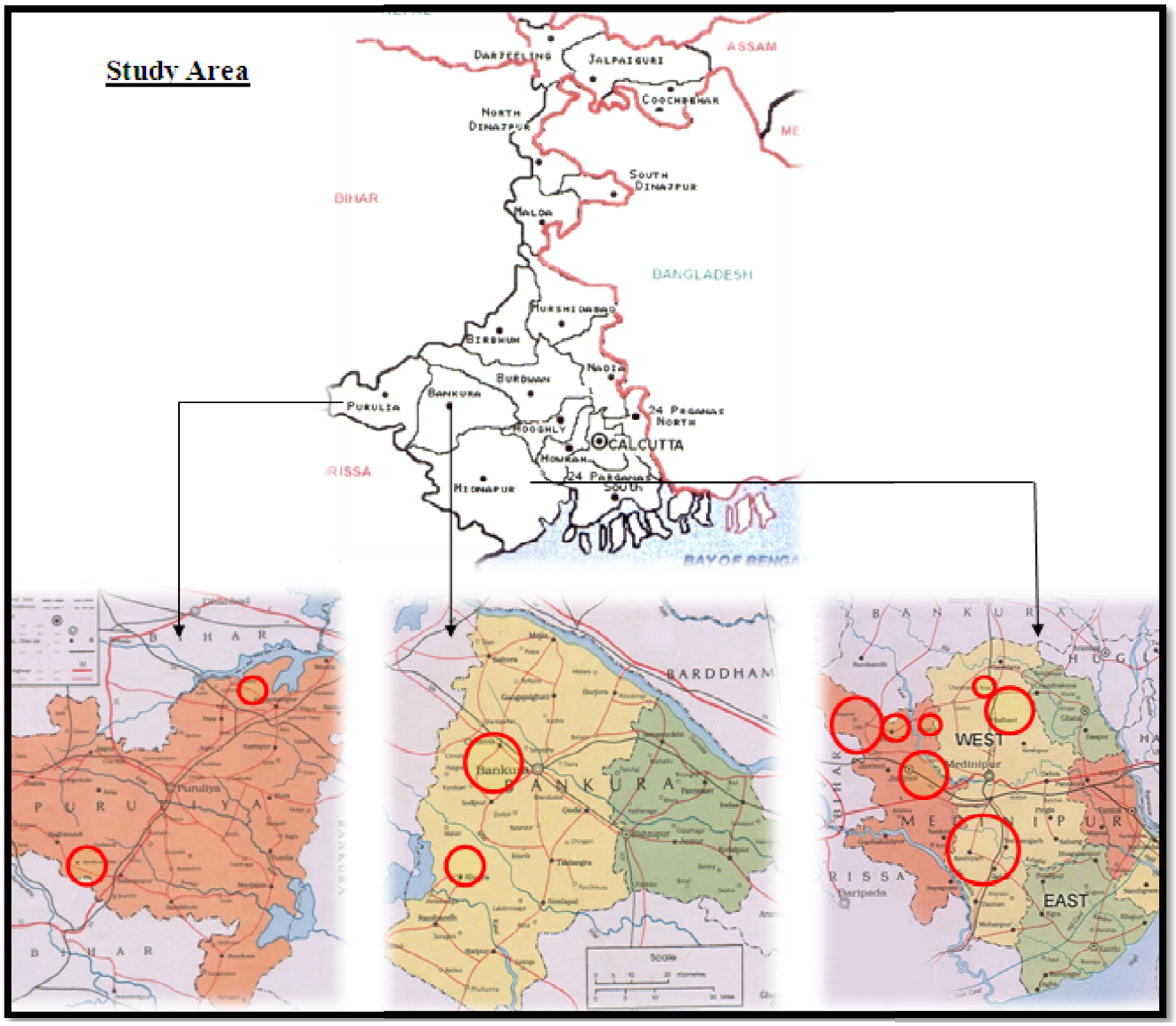
Herbal as health solution has regained its popularity because many studies have substantiated it. A Qualitative analysis was conducted through standardized accepted laboratory technique for screening and study of bioactive compound present in *Loranthus parasiticus* , a hemiparasitic taxa collected from South west Bengal, India. Qualitative phytochemical analysis of this plant confirmed the presence of phytochemicals such as: alkaloids, flavonoids, tannins, terpenoids, reducing sugars, carbohydrates and cardiac glycosides in aqueous extract. This study deals with the significance of these phytochemicals with special reference to their use in traditional medicinal system. Initial study has indicated the significant anti-microbial activity of the leaf extracts of *Loranthus parasiticus* against *Bacillus subtilis, Klebsella pneumoniae, Vibrio cholerae* and *Escherichia coli*.

INTRODUCTION

Now a day, people are too much conscious regarding their health issue. They prefer herbal drug, wherever and whenever, this option is available to them. Herbal drug acts like any other drug, either direct as supplement that support the body system without side effect. The increasing demand of herbal drug develops the herbal industry (*Rahmalia et al*, 2011). Naturally occurring substances used for drugs may be plants, animals and mineral origin. They are organic substances and could be obtained in both primary and secondary metabolic process; they also provide a source of medicine since the earliest time. The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. (Edeoga H.O., Okwu D.E. and Mbaebie B.O.2005) Phytochemicals are the chemical compounds that occur naturally in plants and responsible for color and organoleptic properties. The term is generally used therefore to those chemicals that may have biological significance but are not established as essential nutrients .Scientists estimate that there may be as many as 10,000 different phytochemicals with the potential to cure diseases such as cancer, stroke or metabolic syndrome. (Ganatra *et al.,* 2012) These Antibiotics or antimicrobial properties are because of saponins, glycosides, flavonoids and alkaloids etc are found to be distributed in plants, yet these compounds were not well established due to the lack of knowledge and techniques. (Ajayi I. A., Ajibade O. and Oderinde R. A. 2011) *Loranthus parasiticus* Merr . is a member of Loranthaceae family and is an important medicinal plant with a long history of Chinese traditional use. To date, pharmacological studies have demonstrated significant biological activities, which support the traditional use of the plant as a neuroprotective, tranquilizing, anticancer, immunomodulatory, antiviral, diuretic and hypotensive agent. In addition, studies have identified antimutagenic, antiviral, antihepatotoxic and antinephrotoxic activity. (Moghadamtousi SZ, Kamarudin MN, Chan CK, Goh BH, Kadir HA. 2014).

Study area: A preliminary survey was made in different rural areas of the lateritic zone of South West Bengal from March 2013 to April 2014. The places under the zone of survey included Kushboni forest ($22^0$49'N, $86^0$97'E to $22^0$51'N, $86^0$94'E), Salboni forest ($22^0$61'N, $87^0$21'E to $22^0$64'N, $87^0$25'E), Dhamkurar jungle ($22^0$73'N, $87^0$45'E to $22^0$76'N, $87^0$49'E), Hoomgarh forest ($22^0$82'N, $87^0$23'E to $22^0$83'N, $87^0$25'E), Bulanpur forest ($22^0$73'N, $87^0$10'E to $22^0$74'N, $87^0$11'E). In Purulia District we covered Ajodhya hills ($23^0$13'N, $86^0$06'E to $23^0$27'N, $86^0$19'E), Panchakot hills ($23^0$59'N, $86^0$75'E to $23^0$64'N, $86^0$77'E), and in Bankura district, we visited Sarenga jungle ($22^0$76´N, $87^0$01'E to $22^0$77'N, $87^0$03'E), Jalar jungle ($23^0$03'N, $87^0$06'E to $23^0$08'N, $87^0$07'E).

Corresponding author: Amal Kumar Mondal
UGC-DRS-SAP Department of Botany & Forestry, Vidyasagar University, Midnapore- 721102, West Bengal, India



MATERIALS AND METHODS

Collection and preparation of plant material for extraction

Plant parts were washed with 70% alcohol and then rinsed with sterilized distilled water and air dried. Clean dry plant samples were stored in cotton bags. The materials were homogenized to fine powder with the help of a mixer grinder.

Preparation of acetone extracts

10 g of powdered material was soaked in 30 ml of acetone and kept at 37°C for 24 h on a rotary shaker. After 24 h the previous portion of added acetone was evaporated and the same volume of acetone was again added and placed on a rotary shaker for another 24 h at 37°C. It was then filtered with the help of a Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 minutes. The supernatant was then collected and allowed to evaporate until it was completely dry. The extracts were kept in sterile air tight bottles at 4°C until further use. Before use, 30 mg of dry extract was re-suspended in 1 ml of acetone to make the concentration of the extract was 30 mg/ml (Ushimaru *et al*., 2007).

Preparation of methanolic extracts

10 g of powdered material was soaked in 30 ml of 70% methanol and kept at 37°C for 24 h on a rotary shaker. After 24 h the previous portion of added methanol was evaporated and the same volume of methanol was again added and placed on a rotary shaker for another 24 h at 37°C. It was then filtered with the help of a Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 minutes. The supernatant was then collected and allowed to evaporate until it was completely dry. The extracts were kept in sterile air tight bottles at 4°C until further use. Before use 30 mg of dry extract was re-suspended in 1 ml of 70% methanol to make the concentration of the extract was 30 mg/ml (Ushimaru *et al*., 2007).

Preparation of aqueous extracts

2 g of powdered material of each sample was soaked in 20 ml of distilled water and kept at 37°C for 24 h on a rotary shaker. It was then filtered with the help of a Whatman No. 1

filter paper. The filtrate was centrifuged at 2000 rpm for 10 min. The supernatant was then collected and allowed to evaporate until it was completely dry. The extracts were kept in sterile air tight bottles at 4°C until further use (Das *et al*., 2011).

Bacterial strains

Pure cultures of four bacterial strains *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Vibrio cholerae* were used for the study.

Agar well diffusion

Antimicrobial activity was determined by the agar-well diffusion method. Mueller Hinton Agar was used as media. To standardize the inoculums density for sensitivity test, a Barium Sulphate (BaSO4) turbidity standard, equivalent to 0.5 Mac Farmland standards was used and was cultured on agar medium. Thereafter 6 mm diameter wells were punched in the agar plates. Aqueous and methanolic extracts (100 µl) of the different plant extracts were added to the wells. The plates were then incubated at 37°C for 24 h. After incubation the antimicrobial activity was evaluated by measuring the inhibition zone diameter observed. Each test was performed twice and the average of the results was taken (Ulusoylu *et al*., 2001).

Phytochemical screening: Chemical test were carried out in aqueous extract and on the powdered specimen using standard procedure to identify the constituents as described by Somolenski *et al, 1974* Harborne, 1973 and Sofowora, 1993.

Test for tannins: 1 g of powdered sample was separately boiled with 20 ml in different for five minutes in a water bath and was filtered while hot.1 ml of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10 % ferric chloride were observed for any formation of precipitates and any colour change. A bluish-blackor brownish-green precipitate indicated the presence of tannins.

Test for terpenoids: 5 ml of each extract was mixed in 2 ml of chloroform. 3 ml of concentrated H_2SO_4 was then added to form a layer. A reddish brown precipitate colouration at the interfaceformed indicated the presence of terpenoids.

Test for flavonoids: 1 g of the powdered dried leaves of specimen was boiled with 10 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20 % sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

Test for cardiac glycosides: 5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxysugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

Test for carotenoids: 1 g of sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85 % sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

Test for reducing compounds: To about 1 g of each sample in the test tube was added 10 ml distilled water and the mixture boiled for 5 mins. The mixture was filtered while hot and the cooled filtrate made alkaline to litmus paper with 20 % sodium hydroxide solution. The resulting solution was boiled with an equal volume of Benedict qualitative solution on a water bath. The formation of a brick red precipitate depicted the presence of reducing compound.

Test for alkaloids: 1 g of powdered sample of each specimen was separately boiled with water and 10 ml hydrochloric acid on a water bath and filtered. The pH of the filtrate was adjusted with ammonia to about 6-7. A very small quantity of the following reagents was added separately to about 0.5 ml of the filtrate in a different test tube and observed. Picric acid solution. 10% tannic solution. Mayer's reagent (Potassium mercuric iodide solution).The test tubes were observed for coloured precipitates or turbidity.

RESULTS AND DISCUSSION

The aqueous extract of leaves of *Loranthus parasiticus* were prepared and different tests of phytochemical constituent was performed using generally accepted laboratory technique for qualitative determinations. The study indicated the presence of phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, reducing sugars, carbohydrates and cardiac glycosides. The result of the screening *Loranthus parasiticus* extracts for antibacterial activity is summarized in the Table No.1. *Loranthus parasiticus* exhibited different degrees of antibacterial activities against different strains of bacteria. Aqueous extract of *Loranthus parasiticus* showed inhibition zone of 1 mm only against *Vibrio cholereae*. Methanolic extract of *Loranthus parasiticus* showed inhibition zone of 2 mm against *Vibrio cholereae*, *Eschericheia coli*, and *Klebsiella pneumoniae* each and 5 mm against *Bacillus subtilis* after 24 hrs incubation .The inhibition zone extended to 7mm in case of *V. chloreae* and *B. subtilis* after 48 hrs which remained unchanged in case of others. Acetone extract of *Loranthus parasiticus* showed inhibition zones of 2 mm against *Vibrio cholerae*, *Eschericheia coli and Klebsiella pneumoniae each and* 5 mm against *Bacillus subtilis* after 24 hrs incubation. The inhibition zone extended to 7 mm for both *V cholerae* and *B subtilis* after 48 hrs. but a secondary inhibition zone was also observed in case of *E. coli* and *K. pneumoniae* after 48 hrs which extended to 13 mm and 8 mm respectively .

When a new drug to be discovered, qualitative phytochemical analysis is a very important step as it gives information about the presence of any particular primary or secondary metabolite in the extracts of the plant which is having a clinical significance. The different phytochemicals tests performed on the extracts of *Loranthus parasiticus* leave extracts show the presence of alkaloids, flavonoids, tannins, terpenoids, reducing sugars, carbohydrates and cardiac glycosides. Simultaneously, it is also to be observed that wheather this components have the capacity to suppress any microbes' growth or activity which is already proven by the experiment.

Table 1. Antibacterial activity of leaf extracts of *Loranthus parasiticus* against four bacterial strains

Plants Name	Extract Nature	Bacterial Strains	Inhibition Zone after 24 hrs. (mm)	Inhibition Zone after 48 hrs. (mm)	Control
Loranthus parasiticus	Aqueous	*Vibrio cholerae*	1	1	0
		Escherichia coli	0	0	0
		Bacillus subtilis	0	0	0
		Klebsiella pneumoniae	0	0	0
	Methanolic	*Vibrio cholerae*	2	7	0
		Escherichia coli	2	2 (13)*	0
		Bacillus subtilis	5	7	0
		Klebsiella pneumoniae	2	2 (8)*	0
	Acetone	*Vibrio cholerae*	6	6 (14)*	0
		Escherichia coli	5	8	0
		Bacillus subtilis	2	2 (9)*	0
		Klebsiella pneumoniae	3	11	0

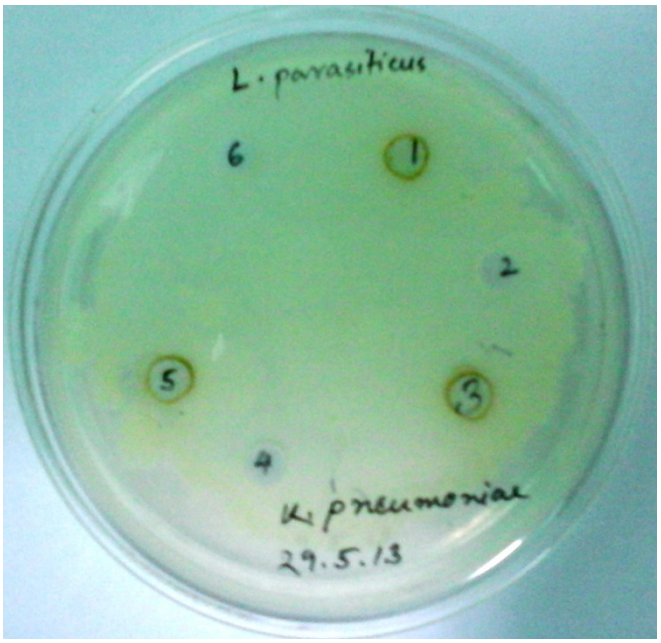


Fig 1. Plate showing antibacterial activity of leaf extract of *L.parasiticus* aginst *Klebsiella pneumoniae*

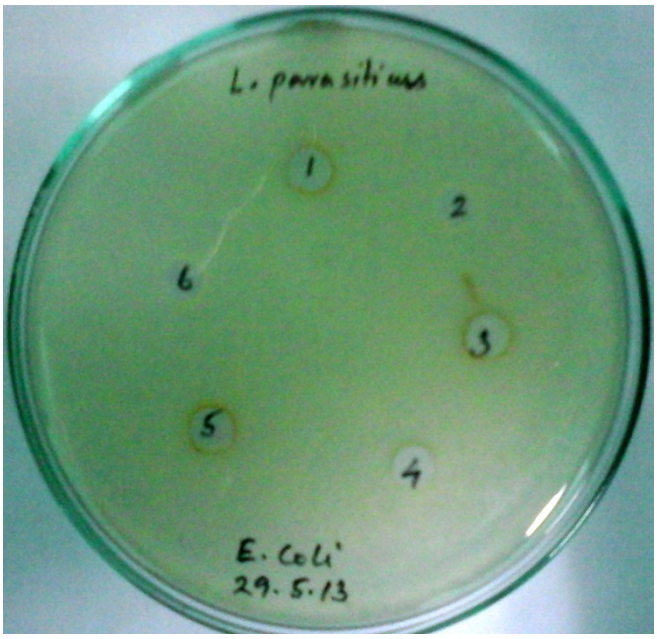


Fig 3. Plate showing antibacterial activity of leaf extract of *L.parasiticus* aginst *Escherichia coli*

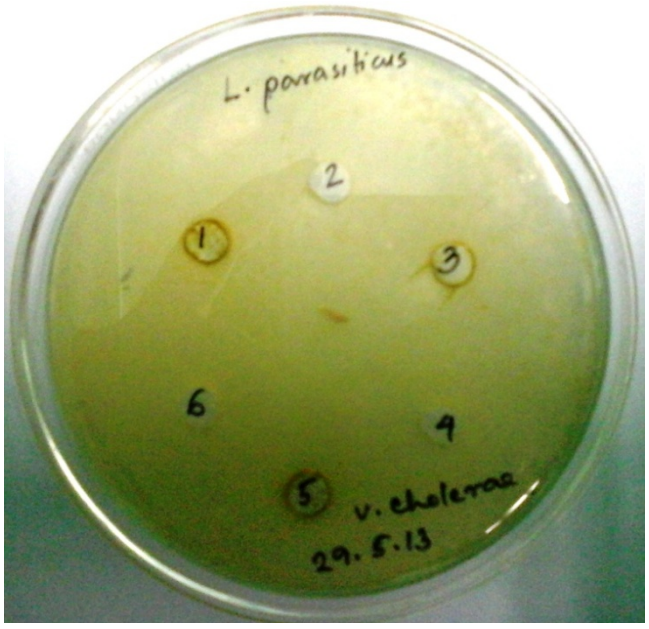


Fig 2. Plate showing antibacterial activity of leaf extract of *L.parasiticus* aginst *Vibrio cholera*

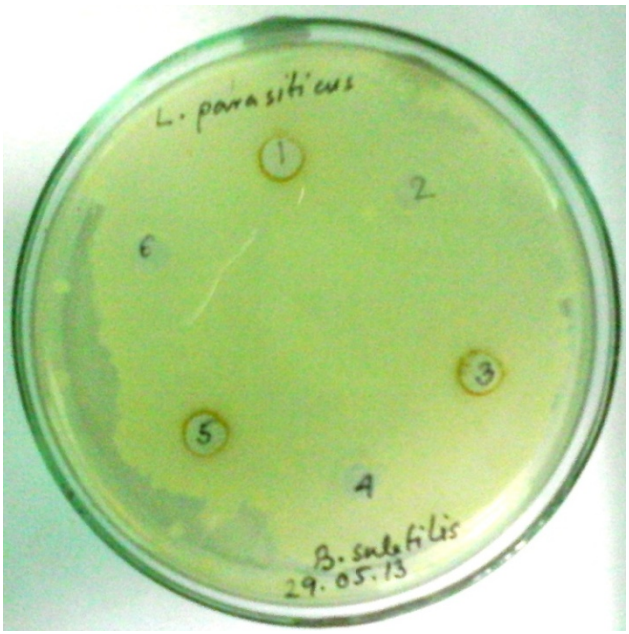


Fig 4. Plate showing antibacterial activity of leaf extract of *L.parasiticus* aginst *Bacillus subtilis*

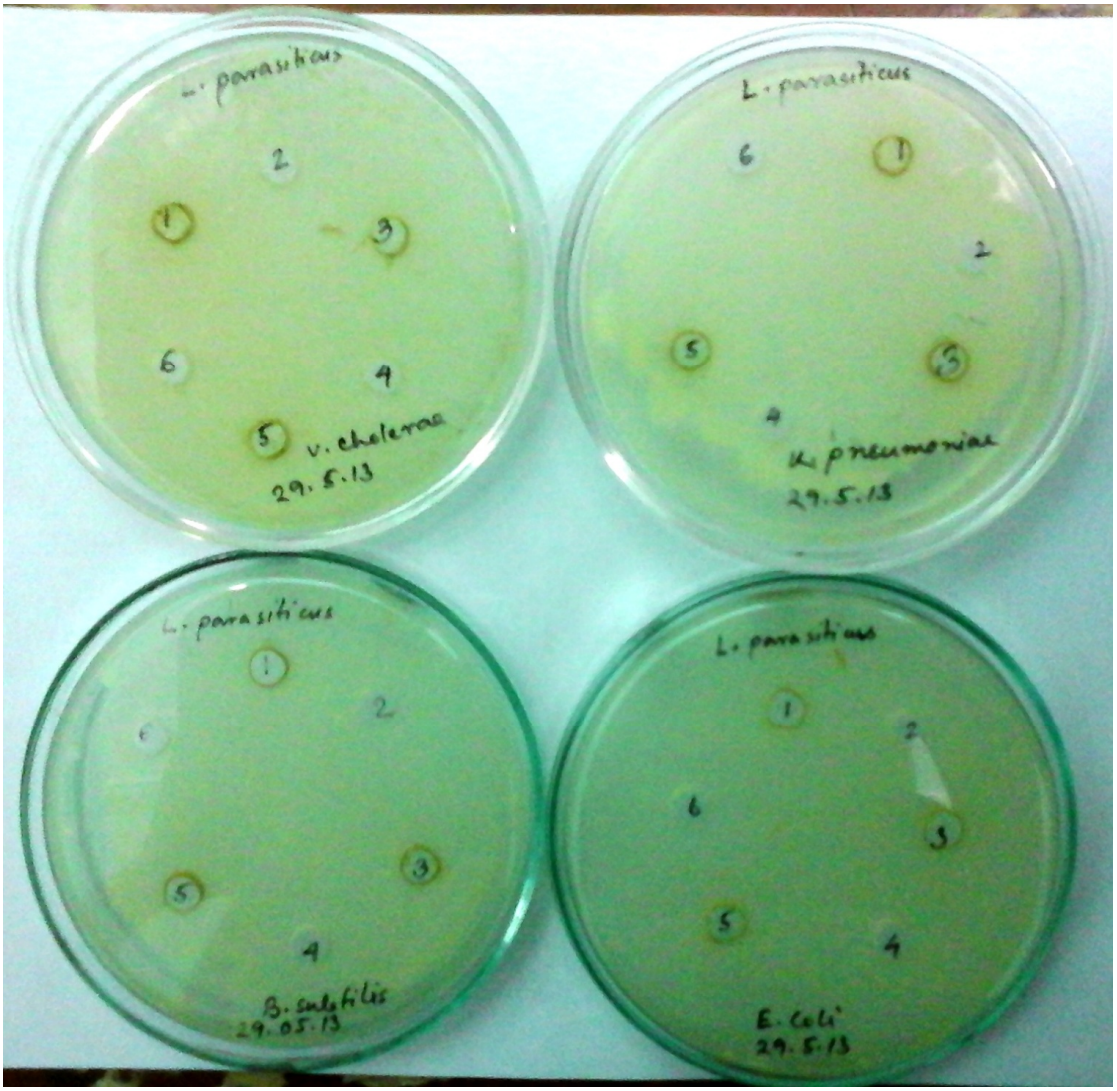


Fig. 5. Four Plates at a glance showing antibacterial activity of leaf extract of *L.parasiticus* aginst *four bacterial strains*

Conclusion

The phytochemical tests performed on the aqueous extracts of *Loranthus parasiticus* leaves shows the presence of alkaloids, flavonoids, tannins, terpenoids, reducing sugars, carbohydrates and cardiac glycosides. All extracts confirm their presence in abundance. Simultaneously, the demonstration of broad spectrum of antibacterial activity by *Loranthus parasiticus* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of gastrointestinal, respiratory tract and dysentery. *Loranthus parasiticus* leaves can be further analyzed for qualitative and quantitative extraction of reported phytochemicals to explore the possibilities of using it as an herb medicine on scientific ground. The effect of these plants on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out.

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