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Antibacterial activity of the rhizome oil extract of *Curcuma caesia* Roxb. against UTI causing bacteria *Escherichia coli*

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Abstract

Plants have medicinal values and serve as potential source of medicine. *Curcuma caesia* Roxb. belonging to the family Zingiberaceae is a less known valuable medicinal plant . In this study the antibacterial activity of rhizome oil extract of *Curcuma caesia* Roxb. was evaluated against UTI causing bacteria *Escherichia coli*. Phytochemical analysis was carried out using GC-MS method. The oil was extracted by hydrodistillation method and antibacterial activity was analysed by Disc diffusion method. Four commercial antibiotics were choosen and compared with Paper disc (whatman no 1 filter paper 5mm) impreganated with oil extract. The oil extract showed a zone of inhibition of 12-13 mm, which is found to be more susceptible than Penicillin-G and Polymyxin-B. The phytochemical analysis by GC-MS showed 34 peaks out of which 8 peaks were studied. The major compound was found to be Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-trans (10.38%). The present study revealed that the oil extract of the rhizome posses antibacterial activity and can be used against UTIs causing bacteria. This antibacterial activity could be possiblely due to the presence of the various compounds.

Key words : Antibacterial activity, UTI, *Curcuma caesia* Roxb, *Escherichia coli*, Zingiberaceae, GC-MS analysis.

Medicinal plants have been used by the people traditionally since centuries. A plant is considered to be medicinal due to the presence of various phytochemicals used against antioxidant, antimicrobial, antipyretic, anti-inflammatory, antitumor activities[5]. Plants are significantly used for prevention, mitigation and cure of several diseases caused by

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different pathogens like fungi, bacteria, viruses etc. Various part of the plants are utilized for medicinal purposes *i.e.*, bulb, gel, leaves, roots, barks, peels rhizome,[14] fruits etc. Researchers have been working on the antibacterial or antifungal components of various medicinal plants due to the increasing use of traditional medicine. More than 400,000 species of tropical flowering plants are known to have medicinal properties and this has made traditional medicine cheaper than modern medicine[17].

Urinary Tract Infections or UTIs are the most common infections caused by bacteria and fungi. The main bacterial organism is the Uropathogenic *Escherichia coli*. This *E. coli* present on the skin or rectum enters through the urethra thus infecting the urinary tract. Nearly 35% of healthy women suffer from the symptoms of Urinary Tract Infection and about 5% of women suffer with the problem of painful urination (dysuria) and frequency per year[8]. There are many antibiotics available for the treatment of UTIs but due to the increasing resistance capacity of the bacteria towards such pharmaceutical antibiotics it became difficult in the treatment of UTIs[12]. Bacteria have the ability to genetically inherit and transmit resistance to drugs[21]. Due to the use of traditional remedies researchers are now frequently searching or studying on plants containing antimicrobial activities. Before using any herbal drug as treatment remedy proper optimization and standardization should be done[6].

Curcuma species belonging to Zingiberaceae family is very well known for their medicinal properties. Various studies have been carried out on the therapeutic properties of *Curcuma* species. Among various *Curcuma* species, *Curcuma caesia Roxb.* also known as black turmeric or black zedoary with dark bluish rhizome[4] is an important species. *Curcuma caesia* Roxb. is a less known, unexplored, non-conventional medicinal plant. It is mainly distributed in the Himalayas, North-East and Central India[11]. The dried leaves and rhizome of *Curcuma caesia* Roxb are used for the treatment of fever, wounds, cancer, leprosy, asthma, piles, allergies, toothache, and other ailments in North-Eastern and Central India[9]. Chewing small amount of rhizomes is used to relieve digestive problems and kidney disorders; however excessive intake of black turmeric may lead to vomiting[1]. The rhizome of black turmeric has also high economic value because of high medicinal value and low cultivation rate. The antibacterial activity of the oil extract for UTI causing organism is not yet explored. This study was carried out in an aim to identify the phytochemical constituents present in oil extract of the rhizomes by GC-MS analysis and observed the potential antibacterial activities of the rhizome oil extract of *Curcuma caesia* Roxb. against UTI causing bacteria *Escherichia coli.*

Selection and collection of plant material :

Curcuma caesia Roxb was selected for the study because of its extensive use by the local people of Northeast India for the treatment of various diseases. Healthy rhizome were used for the study. The rhizome of *Curcuma caesia* Roxb was collected locally from Gogamukh, Tajik Amulapatti (Latitude 27.410307 and Longitude 94.321664) of Dhemaji district Assam. The identification of the plant was confirmed by Departmental

Fig. 1: a) *Curcuma caesia* Roxb plant b) Rhizome c) Inflorescence

Laboratory of Botany, University of Science and Technology Meghalaya, India. [Figure 1(a), 1(b), 1(c)].

Extraction of oil :

The adventitious root of the rhizome were removed and washed thoroughly with tap water to remove dust and other impurities present in it followed by cutting it into fine pieces. The total weight of the rhizome was 500 g. Hydro-distillation technique using a Clevenger apparatus was carried out for the extraction of oil and water was taken as the solvent. It was run at 100° C for 24 hours to achieved a steady distillation. 3.5 ml oil was obtained. This oil was further send for GC-MS analysis.

GC-MS analysis of the oil extract :

The chemical analysis of Oil extract of *Curcuma caesia* Roxb. rhizome extracted at 100°C by all Clevenger type apparatus was done by GC-MS technique. Gas Chromatography – Mass Spectrometry was performed in Laboratory of Central Instrumentation Facility (CIF) University of Science and Technology, Meghalaya. GC-MS analysis was conducted on A Thermo Fisher™ Trace 1300 Gas Chromatograph coupled with single quadrupole Thermo Fisher™ ISQ 7000 Mass Spectrometer. For the separation of the analytes, TG-5MS capillary column (30m x 0.25mm x 0.25µm) was used. Helium was employed as the carrier gas with a flow rate 1.0mL/min. The injector temperature was maintained at 290°C, the MS transfer line temperature at 280°C and the Ion Source Temperature at 260°C. The initial column temperature was kept at 50°C. When the analysis was started, the temperature was raised to 65°C and held for 3.0 mins. A temperature gradient of 3°C/min was set till 280°C and allowed to heat over till end of run time (total runtime 75 min). The MS data was recorded in the full scan mode and mass range m/z 50-650. GC-MS samples were prepared in HPLC grade methanol (~150ppm) and 0.2µL of the sample was injected with a splitless flow of 25 mL/min,giving splitless time around 1 min. Area under the peaks represented relative present of the individual analytes. Chromeleon™ 7.2.10 software was used for the data processing and integrated NIST 2017 Mass Spectral Library for the structural prediction.

Microorganism used :

The Microbial strain used for testing antibacterial activity of the oil extract of *Curcuma caesia* was Gram negative bacteria *Escherichia coli.* The bacterial cultures was

identified and obtained from Duta Deepti Satsang Charitable Hospital, Deogarh, Jharkhand.

Antibiotic susceptibility test :

The test was performed in MacConkey agar using disc diffusion method for the antimicrobial susceptibility testing according to standardized method[3] with slight modifications. A few colonies of overnight cultured organisms *Escherichia coli* were picked with inoculating needle and introduced to a test tube containing 10 ml of sterilized distilled water and mixed thoroughly in vortex mixture to obtained a bacterial suspension. For the sensitivity test the petri plates were filled with MacConkey agar and allowed to solidify. The UV rays were turn-on for 15 minutes to prevent contamination. The bacterial suspension was then introduced to the petri plates using a 1ml micropipette and the plates were gentle move on the surface of the laminar air flow so that the suspension spread uniformly. Paper disc (5mm; whatman no 1 filter paper) dipped in oil extract of *Curcuma caesia* Roxb for 32 hours were gently place at uniform distance using flamed sterilized forcep, these were used as negative control. In the same manner the standard antibiotic disc Penicillin –G(P_1); Streptomycin-S(S_{25}); Polymyxin-B(PB_{50}) and Vancomycin (VA_{10}) were also placed on the plates, these antibiotics were used as positive control. Then the plates were incubated at 37 ± 1 ºC for 24 hours in inverted position. The results were recorded by measuring the zone of inhibition surrounding the disc with antibiotic zone scale.

The rhizome oil of *Curcuma caesia* Roxb. extracted by hydro-distillation method in Clevenger apparatus yielded 3.5ml/500g oil. The GC-MS analysis resulted in a total of 34 peaks out of which 8 peaks were studied. The major constituents were Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-trans (10.38%); Eucalyptol (7.75%); β-Cyclocostundlide (6.44%); c-elemene (4.56%); Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-(1S-(1α,2β,4β))-(4.48%), Camphor(1.65%), Caryophyllene (1.33%), Cyclohexane, 4-ethyl-4-methyl-3-{1-methylethenyl}-1-{1-methylethyl}-{3R-Trans}- (1.14%). The presence of these compounds could be the possible reasons for the antibacterial activity. [Figure 2, Table 1].

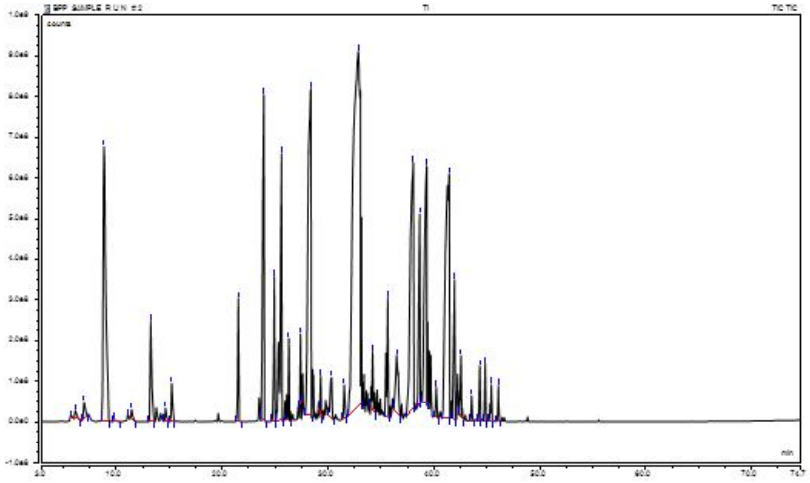

Fig 2 : GC-MS Total ion chromatogram of *Curcuma caesia*

Table-1. Major phytochemical constituents present in oil extracts of *Curcuma caesia* Roxb

Sl no	RT (minutes)	Relative area	Name of the compounds	Molecular formula	Activities
1.	8.83	7.75%	Eucalyptol	$C_{10}H_{18}O$	Helps in gastrointestinal problems. Anti-inflammatory, antioxidant, analgesic, antimicrobial, pro-apototic effect[7]
2.	13.25	1.65%	Camphor	$C_{10}H_{16}O$	Anti-inflammatory and analgesic properties, antiseptic. Cytotoxic activity[20]
3.	21.52	1.14%	Cyclohexane, 4-ethyl-4-methyl-3-{1-methylethenyl}-1-{1-methylethyl}-{3R-Trans}-	$C_{15}H_{24}$	Apoptosis inducer ,anti tumor[23,24]
4.	23.93	4.48%	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methyl-ethenyl)-[1S-(1α, 2β,4β)]-	$C_{15}H_{24}$	Anti-cancer. Anti proliferative effect on cancer cell,[10] apoptosis[25]
5.	24.91	1.33%	Caryophyllene	$C_{15}H_{24}$	Non steroidal Anti-inflammatory, fragrance, metabolites, insect attractant[13]
6.	25.60	4.56%	c- elemene	$C_{15}H_{24}$	Antileishmanial activity, promoting cell membrane damage[16] exhibit good insecticidal properties
7.	28.38	10.38%	Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-trans	$C_{15}H_{20}O$	Might be used as anti-lung adenocarcinoma drug,[22]
8	39.30	6.44%	β-Cyclocostunolide	$C_{14}H_{20}O_2$	Not known

In an earlier study by Pandey and Chowdhury (2003)[18] it was found that the oil extract of *Curcuma caesia* showed 30 peaks in which the major constituent was found to be camphor(28.3%),ar-turmerone (12.3%), (Z)-β-ocimene (8.2%), ar-curcumene (6.8%), 1,8-cineole (5.3%), β-elemene (4.8%), borneol (4.4%), bornyl acetate (3.3%) and c-curcumene (2.82%) where Camphor was found to be the major compound with a relative percent of 28.3%, but in the present study the Camphor content was only 1.65% which was comparatively very less.

From the antibiotic susceptibility test it was found that the oil extract of *Curcuma caesia* Roxb rhizome were susceptible to the *Escherichia coli*. This finding was performed by Disc diffusion method. [Figure 3]. It was observed that the oil extract showed a zone of inhibition of 12mm and 13mm which indicate susceptibility to *Escherichia coli* [Table 2]. Hence this study brought out that *Curcuma caesia* Roxb, could be probably use in treating bacterial infections like UTIs since it has antibacterial properties and can be used in formulating drugs. Standard antibiotics such as Penicillin –G(P_1); Streptomycin-S(S_{25}); Polymyxin-B(PB_{50}) & Vancomycin (VA_{10}) were used to compare with the paper disc (whatman No. 1 Filter paper) impregnated with the extracted oil. The antibiotic Penicillin-G and Polymyxin-B showed a zone of inhibition of 0mm and 0.8mm respectively which indicates its resistance to *Escherichia coli*. Streptomycin and Vancomyxin showed a zone of inhibition of 17mm and 15mm respectively which indicates that it is susceptible to *Escherichia coli*. [Table 3].

Table-2. Zone of inhibition of oil extract

Sl No	Plate no.	Zone of Inhibition (mm)
1	b	12 mm
2	c	13 mm

Table-3. Zone of inhibition of antibiotic and their interpretation

Sl no	Antibiotics	Zone of inhibition in mm	Interpre-tation
1	Penicillin G	0mm	Resistant
2	Streptomycin S	17mm	Susceptible
3	Polymyxin B	0.8mm	Resistant
4	Vancomycin	15mm	Susceptible

In a previous report it was found that the ethanolic extract of *Curcuma caesia* Roxb have some shown antibacterial activity[9]. Similarly a work done earlier showed that the methanol, Acetone and Chloroform extract of the rhizome have efficacy against *E. coli* and other gram negative bacteria *P. vulgaris* and *K. Pneumoneae*[19]. The present work have shown that oil extract of *Curcuma caesia* Roxb rhizome have activity against *Escherichia coli*. But according to earlier studies less information is available regarding the clinical toxicity and phytoanalytical properties[2].

It was revealed that the methanolic rhizome extract of different genotypes of *Curcuma caesia* Roxb showed only efficiency against gram positive bacteria and does not have efficacy against gram negative bacteria[15]. Whereas in the present study the oil extract showed efficacy against gram negative bacteria *Escherichia coli.* This could be the

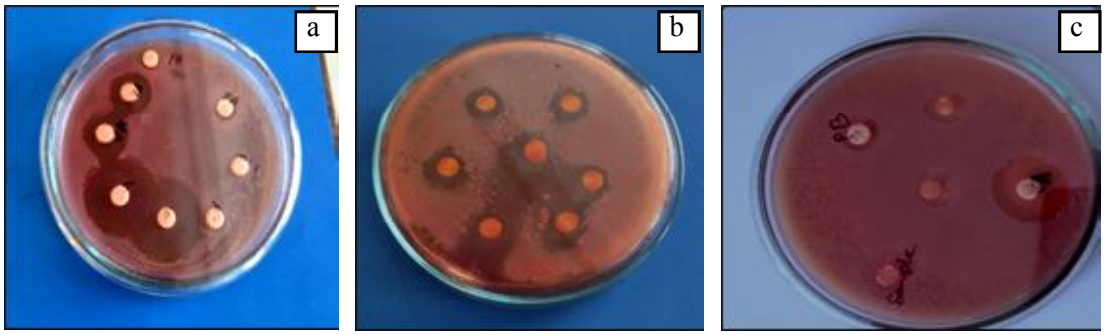

Fig. 3. Antibiotic susecptibility test showing zone of inhibition of the oil extract of *Curcuma caesia* Roxb against *E. coli*: (a)- standard antibiotic (penicillin B, Streptomycin S, Polymyxin B and Vancomyxin), (b) –oil extact, (c) oil extract in the middle column and polymyxin in the either sides

due to the bioactive constituents which may vary depending on its geographical distributions.

In the present study the results revealed that oil extract of *Curcuma caesia* Roxb rhizome showed significant antibacterial activity against UTI causing gram negative bacteria *Escherichia coli*. The compounds found in the oil extract could be the potential source of antibacterial activity. Hence this study showed that the oil extract of *Curcuma caesia* Roxb. rhizome can be used for the treatment of UTIs causing bacteria and can also be used as a natural and effective alternative to antibiotic. This study also validates its used as traditional medicines by the local peoples. However further studies should be carried out to find out the potential phytochemical compound responsible for its effect.

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