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Research Article

SEPARATION OF ACTIVE COMPOUNDS FROM *CURCUMA CAESIA* ROXB. AND *CURCUMA AROMATICA* SALISB. OF ASSAM, INDIA BY TLC- BIO AUTOGRAPHY AND ANTIBACTERIAL ASSAY OF SEPARATED EXTRACTS

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ABSTRACT

Curcuma caesia Roxb. and *Curcuma aromatica* Salisb. are two widely used ethnomedicinal plants under the genus Curcuma of Zingiberaceae. These plants have wide application in the preparation of traditional medicines by different ethnic communities. In the present study attempt has been made to separation of the rhizome extracts of *Curcuma caesia* and *Curcuma aromatica* by TLC and bio autography of separated extracts in order to isolate the most active fraction of the crude extracts. TLC was performed with Ethyl acetate crude extracts of rhizome of both plants. Fractions were isolated from TLC plate and allowed for contact bio autography. Re-chromatography of active bands was carried out. The process is repeated for several times in order to separate the most active fraction, resulting isolation of two most active bands for both the plants which possess highest antibacterial activity. The result clearly indicates the presence of biologically active fraction in the rhizomes of these plants and validates their ethnomedicinal claims.

Keywords: TLC, Bio autography, *Curcuma*, Antibacterial, Ethnomedicine.

INTRODUCTION

The family Zingiberaceae is well known for its immense medicinal values are distributed widely throughout the tropics, particularly in Southeast Asia[1]. North eastern India is one of the richest sources of Zingiberene with about 19 genera and 90 species. Most of the Zingiberaceae members are found here at wild states and many of them are lack behind of scientific investigation. *Curcuma, Zingiber, Alpinia, Kaempferia, Hedychium, Elettaria, Costus* etc are the most important genus under the family Zingiberaceae. The genus *Curcuma* of Zingiberaceae has high medicinal potential, most of the species under the genus *Curcuma* has been widely used by different ethnic communities against several common ailments. The genus *Curcuma* in Assam is represented by *Curcuma longa* Linn., *Curcuma aromatica* Salisb., *Curcuma amada* Roxb., *Curcuma angustifolia* Roxb., *Curcuma caesia* Roxb. etc. *Curcuma caesia* Roxb. and *Curcuma aromatica* Salisb has wide application in the preparation of traditional medicines by different ethnic communities.

Traditionally the rhizomes of *Curcuma caesia* Roxb. are used in the treatment of hemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wound, vomiting, menstrual disorder, smooth muscle relaxant activity [2], anthelmintic, aphrodisiac, inflammation, gonorrheal discharges, etc.[3]. The dried rhizome powder is mixed with powdered seeds of *Andrographis paniculata* and applied during insect, scorpion and snake bite as anti-inflammatory. Fresh rhizome juice along with mustard oil is given daily in asthma and dysentery. Rhizome paste is used for healing wound[4]. Dried rhizome and leave of the plant are used in piles, leprosy asthma, cancer, wounds, tooth ache, vomiting, and allergies[5,6]. The Khamti tribe of Lohit district of Arunachal Pradesh applied the paste of fresh rhizome in case of snake and scorpion bite[7]. The crushed rhizome paste is applied against cut or injury to control bleeding and quick healing[8]. The rhizome is administered during inflammation of tonsils[9]. Similarly rhizomes of *Curcuma aromatica* Salisb., is used by different ethnic communities in indigenous medicine for external applications on skin diseases, Itches[10], Sprain[11], antidote for snake and insect bite, in dysentery and stomach-ache[4] and for Constipation[12] in Malaria,[13]. Khasi and Garo tribes of Meghalaya use the paste of rhizomes as anthelmintic[14].

Some work has been carried out on *Curcuma longa* and *Curcuma amada* from this region, but *Curcuma caesia* and *Curcuma aromatica* are two widely used medicinal plants found in wild state is very less attended[15]. Phytochemical investigations of these plants are important to know the drug potential of these species. In addition, the knowledge would further help to understand the value of folk remedies. Screening for plant active components required separation of plant extracts and bio assays of separated extracts. Paper or thin-layer chromatography is the common method for separating the components of a plant extract. TLC is less time consuming, low cost and can be performed with less complicated technique it has a wide application in pharmaceutical analysis. In the present study attempt has been made to Separation of the extracts of *Curcuma caesia* and *Curcuma aromatica* by TLC and Bio autography of separated extracts.

MATERIALS AND METHODS

Collection of Plant Material

The fresh rhizome of *Curcuma caesia* Roxb. and *Curcuma aromatica* Salisb were collected from three districts of Upper Brahmaputa Valley of Assam, India viz. Sivasagar, Jorhat and Golaghat in the month of September, 2018. The plant material was identified and authenticated in the department of Botany, J.B. College (Autonomous), Jorhat, Assam, India. The rhizome was then washed carefully to remove all the dirt and cut into small pieces and was shade dried separately. The well dried rhizome pieces were then grounded to fine powder using a mixer grinder and preserved separately in airtight containers with proper labelling for future use.

Preparation of Extracts

In brief, 10 grams of each plant material was weighted, mixed with 100 ml of ethyl acetate and agitated for 48 h at 30 °C and 150 rpm. After incubation the extract was collected and fresh extraction solution (50 ml) was added to the flask and incubated in same conditions for another 24 h. Extracts were pooled and filtered through Whatman no. 1 filter paper. The extracts ware dried in Soxhlet apparatus and dissolved in suitable amount of solvents.

Preparation of TLC Plates

The TLC plates were prepared by using Silica gel 'G' as 30 gm of silica gel was weighted and made to a homogenous suspension with 60 ml distilled water for two minutes, this suspension was distributed over the plate which was air dried until the transparency of the layer disappeared. The plates were dried in hot air oven at 110°C for 30 min and then stored in a dry atmosphere and used whenever required.

TLC was performed with crude ethyl acetate extract, with gradient polarity of solvents using silica gel. 50 µl extract from each sample was spot on the TLC plate 2 cm above its bottom with the help of capillary tubes and dried. TLC chamber was filled with 75-80 ml of the solvent system and TLC plate was allowed to run for about 30 min. Plate was removed from the chamber and solvent front was marked immediately with a pencil for calculate of Rf. Solvent was dried up from the plate and visualized under the UV/ Fluorescent lamp and active spots were marked. Bands were scraped from TLC plate and collected in fresh tube. After that these bands (silica gel contains band) were dissolved in methanol and centrifuged at 1200 rpm for 5 minutes. After, those supernatants were transferred in fresh tube; this process was repeated 2 times. Finally, supernatant was allowed to dry and store at 4°C for further analysis.

Extraction procedure

TLC was performed with Ethyl acetate crude extracts (EA). The fractions or bands on TLC plate were scrapped. Antimicrobial activity assay was carried out with the materials of these scrapped bands and band showing highest antimicrobial activity was selected for re-chromatography using gradient polarity of solvents on silica gel. The solvents were Hexane: ethyl acetate (1:1), Water: methanol: Ethyl acetate (0.5:1.5:8), Ethyl acetate: methanol: acetic acid (7:1:2), toluene: ethyl acetate (4:1), benzene: ethyl acetate (5:1). This process was continued for several times so that active fraction can be separated by TLC. Hexane: ethyl acetate (1:1), Water: methanol: Ethyl acetate (0.5:1.5:8), Ethyl acetate: methanol: acetic acid (7:1:2), toluene: ethyl acetate (4:1), benzene: ethyl acetate (5:1)

Autobiography/Antimicrobial activity assay

For antibacterial activity assay bacterial strains *Bacillus subtilis, E. coli* and *Enterococcus faecalis* were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. The strains were maintained on nutrient agar slant and kept in refrigerator. Contact bio autography was carried out following the Sasidharan *et al*.,[16] Kagan and Flythe[17] with slight modification by following zone inhibition method. The MHA (Muller Hilton's Agar) plates were spread inoculated with 100 µl of log cultures of all the bacteria followed by placing the discs containing concentration 1000 µg of samples. One disc was loaded with solvent (ethyl acetate) alone which served as vehicle control and ciprofloxacin solution (10 µg/disc) was taken as positive control. The plates were incubated at 30 °C for 16-24 h and halo zones created around the discs were measured and recorded.

RESULT

TLC was performed with Ethyl acetate crude extracts (EA). Three bands (a, b and c) for *Curcuma caesia* (CC) and two band (a and b) *Curcuma aromatica* (CA) were collected (Figure 1). Antimicrobial activity assay was carried out with the materials of these scrapped bands and band showing highest antimicrobial activity was 'b' for both *Curcuma caesia* and *Curcuma aromatica*. These bands were selected for re-chromatography. Again, four bands (A, B, C and D) were scrapped and collected for both *Curcuma caesia* and two bands *Curcuma aromatica* (Figure 2). Antimicrobial activity assay was carried out with the materials of these scrapped bands and band "B" of *Curcuma caesia* and band "C" of *Curcuma aromatica* were found most active against bacterial strain. Re-chromatography of these bands' "B" of *Curcuma caesia* and band "C" of *Curcuma aromatica* were carried out (Figure 3). In this way chromatography was carried out for seven times in order to isolate the active fraction of the plants by TLC. Finally, five bands (Ca, Cb, Cc, Cd, Ce) were scrapped and collected for *Curcuma caesia* and five bands (Aa, Ab, Ac, Ad, Ae) were scrapped and collected for *Curcuma aromatica* through subsequent bioassay guided isolation (Figure 5)

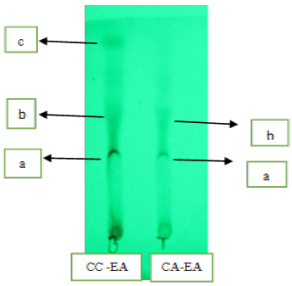


Figure 1: TLC with Ethyl acetate extract (EA)

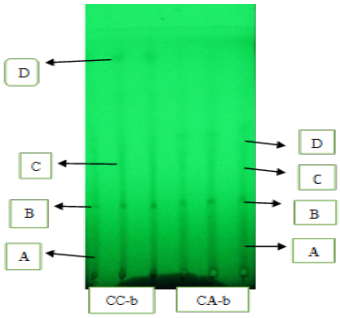


Figure 2: TLC with active band "b" isolated from EA extracts after bio autography

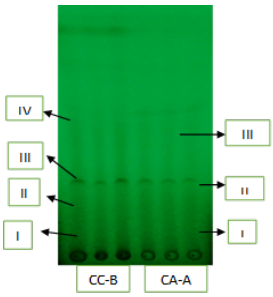


Figure 3: TLC with active band "B" of *Curcuma caesia* and band "A" of *Curcuma aromatica* isolated from band "b" extracts through bio autography

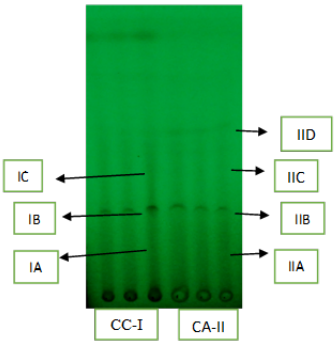


Figure 4: TLC with active band "I" of *Curcuma caesia* and band "II" of *Curcuma aromatica* isolated from band "B" of *Curcuma caesia* and band "A" of *Curcuma aromatica* through bio autography

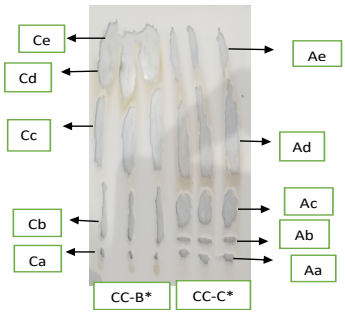
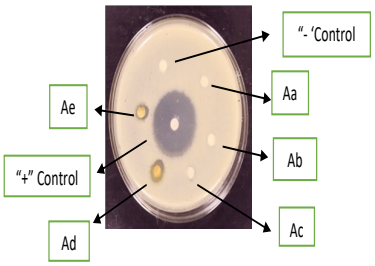


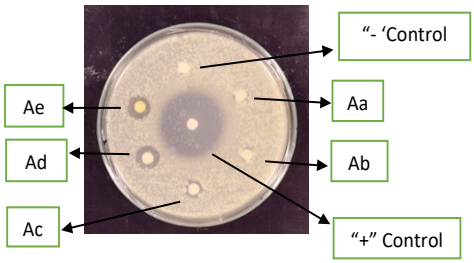
Figure 5: TLC plate after scrapping bands for bio autography

Antibacterial activity assay of Scrapped bands

Antimicrobial activities of samples were checked by following zone inhibition method. Scrapped band Cd and Ce of *Curcuma caesia* has antibacterial potency against all the three-test bacterial strain. Similarly, Ad and Ae of *Curcuma aromatica* has shown measurable antibacterial activity against all the three-test bacterial strain. However, the band Cd in case of *Curcuma caesia* and band Ae in case of *Curcuma aromatica* exhibited highest zone of inhibition (Table 1 and 2)

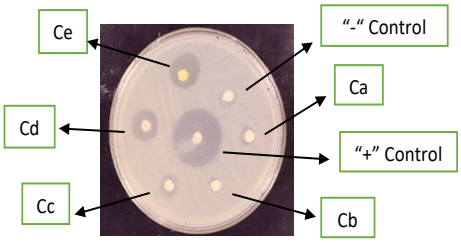


A

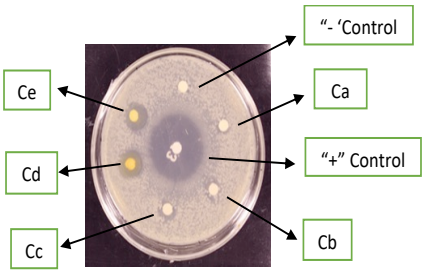


B

Figure 6: Zone of Inhibition test of isolated fraction of *Curcuma caesia* against test bacterial strain *Bacillus subtilis* (A) and *E. coli* (B)



A



B

Figure 7: Zone of Inhibition test of isolated fraction of *Curcuma aromatica* against test bacterial strain *Bacillus subtilis* (A) and *E. coli* (B)

Table 1: Zone of Inhibition (in mm) of isolated fraction of *Curcuma caesia* against test bacterial strain

Test Organism	ZOI in mm						
	Sample Ca	Sample Cb	Sample Cc	Sample Cd	Sample Ce	"+" Control	"-" Control
B. subtilis	-	-	7	13	15	30	-
E. coli	-	-	8	13	13	32	-
B. faecalis	-	-	-	9	11	30	-

Table 2: Zone of Inhibition (in mm) of isolated fraction of *Curcuma aromatica* against test bacterial strain

Test Organism	ZOI in mm						
	Sample Ca	Sample Cb	Sample Cc	Sample Cd	Sample Ce	"+" Control	"-" Control
B. subtilis	-	-	-	11	10	31	-
E. coli	-	-	8	12	12	32	-
B. faecalis	-	-	-	9	7	30	-

DISCUSSION

Thin-layer chromatography is a widely used method for separating the components of a plant extract. Thin-layer chromatography coupled with bio autography is helpful for subsequent Bioassay guided isolation of biologically active fraction of plant extract. Phytochemical screening of the rhizome crude extract of the plants in different solvents exhibits positive test for different phyto constituents like tannin, phenols, flavonoids, alkaloids, terpenoids, saponins, amino acids etc in both the plants. The antibacterial activity of crude extract of rhizome of *Curcuma caesia* and *Curcuma aromatica* was found to be maximum in ethyl acetate extract followed by methanolic extract with significant zone of inhibition against test bacterial strain. Therefore, in order to isolate the most active fraction of these plants Thin-layer chromatography and contact bio autography of ethyl acetate extracts were carried out, which is resulting in the isolation active antibacterial fraction of these plants. The result clearly indicates the presence of biologically active fraction in the rhizomes of these plants which are effective against both gram negative (*Escherichia coli*) and gram positive (*Bacillus subtilis, Enterococcus faecalis*) microbe.

CONCLUSION

Curcuma caesia Roxb. and *Curcuma aromatica* Salisb. are two important ethno medicinal plants of Zingiberaceae. The plants have wide application in the preparation of traditional medicines by different ethnic communities. Present study reveals that thin-layer chromatography with bio autography is helpful for isolation of active fraction of these plants, which may be helpful for identification of active principles from the plants. The data generated from antimicrobial activity assay of the isolated fractions clearly indicates about the medicinal potency of these plants and also validates the ethno medicinal claims of different ethnic groups regarding use of these plants as ethno medicines.

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REFERENCES

1. Rasool S, Maqbool M. An overview about *Hedychium spicatum*: a review, Journal of Drug Delivery and Therapeutics 2019; 9(1-S): 476-480.
2. Karmakar I, Dolai N, Bala A, Saha P, Sarkar N, Haldar PK. Scavenging activity of *C. caesia* rhizome against reactive oxygen and nitrogen species. Oriental Pharmacy and Experimental medicine, Springer; 2011. p. 221-228.
3. Arulmozhi DK, Sridhar N, Veeranjaneyulu A, Arora SK. Preliminary mechanistic studies on the smooth muscle relaxant effect of hydroalcoholic extract of *Curcuma caesia*. Journal of Herbal Pharmacotherapy 2006; 6: 117-24.
4. Tushar, Basak S, Sarma GC, Rangan, L. Ethno medicinal uses of Zingiberaceous plants of Northeast India, Journal of Ethno pharmacology 2010; 132: 286–296.
5. Israr F, Hassan F, Naqvi BS, Azhar I, Jabeen S and Hssan SMF. Studies on antibacterial activity of some traditional medicinal plants used in folk medicine, Pakistan Journal of Pharmaceutical Sciences 2012; 25(3): 669- 674.
6. Syamkumar S and Sasi Kumar B. Molecular marker based genetic diversity analysis of *Curcuma* species from India. Scientia Horticulturae 2007; 112(2): 235-241.
7. Kagyung R, Gajurel PR, Rethy P and Sing B. Ethno medicinal plant used for gastrointestinal diseases by Adi tribe of

Dehang-Debang biosphere reserve in Arunachal Pradesh, Journal of Traditional Knowledge 2010; 9(3): 496-50
8. Trivedi PC. Ethno medicinal plant of India. Aviskar Publisher, Jaipur; 2003. p. 46.
9. Mia MK, Kadir MF, Hossan S and Rahmatullah M. Medicinal plants of the Garo tribe inhabiting the Madhupur forest region of Bangladesh, American-Eurasian Journal of Sustainable Agriculture 2009; 3(2): 165-171.
10. Majumdar K and Datta BK. A study on ethno medicinal usage of plants among the folklore herbalists and Tripuri medical practitioners: Part-II Natural Product Radiance 2007; 6(1): 66-73.
11. Buragohain J, Ethno medicinal Plants Used by the ethnic Communities of Tinsukia District of Assam, India Recent Research in Science and Technology 2011; 3(9): 31-42
12. Das AK, Dutta B, Sharma GC. Medicinal plants used by different tribes of Cachar district, Assam, Indian Journal of Traditional Knowledge 2008; 7 (3): 446-454.
13. Paul S, Devi N, Sarma G. Ethno botanical utilization of some medicinal plants by Bodo peoples of Manas Biosphere Reserve in the treatment of malaria. International Research Journal of Pharmacy 4(6): 102-105. 10.7897/2230-8407.04622.
14. Saleem M, Daniel B, Murali K. Antimicrobial activity of three different rhizomes of *Curcuma longa* and *Curcuma aromatica* on uropathogens of diabetic patients. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(4): 273-279.
15. Das K and Saikia LR. Phytochemical Analysis of *Curcuma caesia* Roxb. and *Curcuma aromatica* Salisb. of Upper Brahmaputra Valley, Assam, India, International Journal of Pharmacy and Biological Sciences 2018; 8 (2): 775-781
16. Sasidharan S, Chen Y, Saravanan D, Sundram K M, Yoga Latha L. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts; African Journal of Traditional, Complementary and Alternative Medicine 2011; 8(1): 1–10.
17. Kagan IA, Flythe MD. Thin-layer Chromatographic (TLC) Separations and Bioassays of Plant Extracts to Identify Antimicrobial Compounds. Journal of visualized experiments 2014; (85). PMC4158999

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