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HPTLC profiling and antimicrobial studies on *Curcuma aromatica* Salisb and *Madhuca longifolia* (Koenig).

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ABSTRACT

Curcuma aromatica and *Madhuca longifolia* are the medicinal plants commonly used by the tribal peoples of Kinwat and Mahur forest range for the treatments of various ailments. In the present investigation these plants were tested for antimicrobial activities and found that, they have considerable antibacterial potential against tested bacteria. HPTLC analysis of rhizome and flower of both the medicinal plants showed different phytochemical compounds with variable RF values and concentration. *C. aromatica* rhizome showed three polyvalent phytochemical compounds with RF value ranging from 0.37 to 0.91. flower extract of *M. longifolia* showed nine compounds with RF value ranging from 0.02 to 0.91. The HPTLC obtained using tolune, ethyl acetate and formic acid in the current study will be very much useful in the correct identification of the drug and can be used for finding out any type of adultrations.

Keywords: *Curcuma aromatica, Madhuca longifolia,* HPTLC, Antibacterial.

INTRODUCTION

Medicinally important plants present around us are of great importance in primary healthcare of individual and society in many developing countries. It is said that medicinal plants are nature's gift to human being to make life disease free and healthy (Jadhav, 2018). In present scenario world health organization is taking official interest in this to develop traditional system of healthcare where special attention has been given on folk medicine as safety for microbial and non-microbial diseases (WHO, 1978). Herbal medicines are the major remedy for thousands of years and have made great contribution to maintain human health in many parts of the world in the rural areas of developing countries as primary source (WHO,1993).

About 80% of the population in various developing countries depend on traditional medicine for human alleviation due to its fewer side effects. It is the property of most of the plant-based drugs to be simple, effective and offering a broad spectrum of activity with greater emphasis on preventive action. In addition to that large number of secondary metabolites is also produced by some of the higher plants (Annalakshmi *et. al.* 2013).

MATERIALS AND METHODS

Collection and identification of plant material:

The plants were collected during ethnomedicinal survey of study region in the year 2020-21. The collected plants brought to the laboratory and preserved inform of herbarium. These plant species were identified with the help of floras. (Cooke, 1967; Almeida, 1990; Naik, 1998, Yadav-Sardesai, 2002).

Preparation of extracts:

The plant material in the form of flowers, fruits and tubers were collected from the forest and brought to the laboratory. They were cut into small pieces and wash thoroughly with tap water to remove the contaminants and dried under shade for about 8-10 days separately. The dried materials can be grind into fine powder and store in airtight containers at room temperature till the extraction. The crude extracts will be prepared by extracting 10gm. flowers, fruits and tubers powder with 100m of methyl alcohol by soxhlet extractor for about 90-120 minutes separately. These extracts were used for antibacterial studies.

Antimicrobial studies:

Antibacterial activity of different pathogenic bacteria were carried out by well plate diffusion method. The inoculum of the microorganism was prepared from the bacterial culures. 15ml of nutrient agar (Hi media) medium was poured in clean sterilized petri plates and allowed to cool and solidify. 100 μl of broth of bacterial strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly. Wells of 6mm in diameter were bored using a sterile cork borer. Solution of all the compounds (1mg/ml) in DMSO were prepared. 100ul of plant extracts solutions was added to the wells. The petri plates were incubated at 37^0C for 24h. Streptomycin (1mg/ml) was prepared as a positive control and DMSO was taken as negative control. Antibacterial activity was evaluated by measuring the diameters of the zone of inhibition is (ZI) and all the determination were performed in triplicates.

HPTLC analysis of extracts:

HPTLC fingerprints are made in the manner described by Annalakshmi *et. al.* (2013) and Jadhav (2018) with minor modifications. Two microliters of ethanolic extracts (belt length -6.0 mm) were used on the pre-fabricated TLC sheet of silica gel G60 F254 for a 200 µm- 05 x10cm thick plate (Merck, Mumbai) using Linomat V. The TLC applicator (Camag, Muttenz, Switzerland) is fitted with a 100-µL syringe. Prior to application, the plate was pre-washed with methanol AR and dried at 60 ° C. TLC plates are developed using the cellular category Toluene: ethyl acetate: Formic Acid (2.5: 2.0: 0.3) in the trough chamber of the Camag HPTLC (10x10cm). The chamber was saturated with filter paper for 15 minutes and plate was run for 10 minutes. The plate is built up to 85.0 mm and dried under air flow. Split bands are measured with HPTLC densitometric scanner using Camag TLC Scanner 4 in absorption mode using Win CATS software (version 1.4.8). After scanning the spectra and the obtained tables are analyzed to interpret the results.

RESULT AND DISCUSSION

HPTLC: Because of extreme complex analytes, the analysis of herbs and herbal preparations is more challenging. Therefore, HPTLC offers many advantages in this regard. HPTLC finger printing is a valuable quality assessment tool for the evaluation of botanical materials, it allows for the analysis of a broad number of compounds both efficiently and cost effectively. HPTLC studies have shown that it is more versatile than ordinary TLC methods as the spots are well resolved. The HPTLC method is simple, rapid, accurate, reproducible, selective and economic, can be used for quality control analysis and for quantitative determination of the plant material (Palani and Natesan, 2011).

For the analysis of herbs and herbal preparations, the HPTLC technique is especially suitable for comparison of samples based on fingerprints and for conveniently performing quantitative determinations based on scanning densitometry. Fingerprint analysis by HPTLC is one of the most powerful tools to link the botanical identity to the chemical constituent profile of the plant.

In combination with microscopic investigations, the fingerprint provides the means for a convenient identity check. It can also be used to detect adulterations in raw materials. From the constituent profile, a number of marker compounds can be selected, which might be used to further describe the quality of the herb or the herbal preparation. HPTLC can also be employed for quantitative determination of such marker compounds (Mukherjee, 2019).

Results of HPTLC profile of *Madhuca longifolia* flower extract recorded at 366 nm showed presence of nine polyvalent compounds (Fig 1) with ascending Rf values 0.02 to 0.91. From the chromatogram and Rf table (Table 1) it is clear that the compound number forth, second and fifth has highest concentration i.e. 48.69%, 18.66% and 11.77 with 0.21, 0.09 and 0.38 Rf values respectively.

Similarly, the results of HPTLC profile of *Curcuma aromatica* rhizome extract recorded at 366 nm showed presence of three polyvalent compounds (Fig 2) with ascending Rf values 0.37 to 0.91. From the chromatogram and Rf table (Table 2) it is clear that the compound number third, first and second has highest concentration i.e. 39.86%, 35.35% and 24.79% with corresponding Rf values 0.91, 0.37 and 0.78 respectively. The corresponding densitometric photo plates are shown in fig 3.

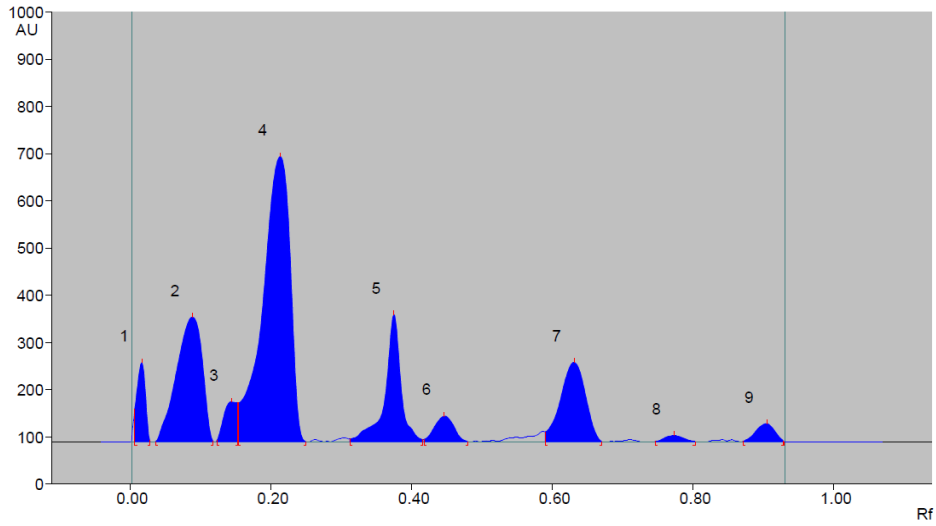

Fig1: HPTLC chromatogram of *Madhuca longifolia* flower extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	38.9 AU	0.02 Rf	167.3 AU	10.06 %	0.03 Rf	2.7 AU	1635.3 AU	3.82 %
2	0.04 Rf	0.1 AU	0.09 Rf	264.0 AU	15.87 %	0.12 Rf	1.1 AU	7981.4 AU	18.66 %
3	0.12 Rf	2.2 AU	0.14 Rf	84.4 AU	5.07 %	0.15 Rf	11.8 AU	1313.9 AU	3.07 %
4	0.15 Rf	31.8 AU	0.21 Rf	603.9 AU	36.31 %	0.25 Rf	0.3 AU	19540.9 AU	45.69 %
5	0.31 Rf	5.6 AU	0.38 Rf	269.5 AU	16.21 %	0.42 Rf	4.5 AU	5031.7 AU	11.77 %
6	0.42 Rf	4.6 AU	0.45 Rf	54.1 AU	3.25 %	0.48 Rf	0.1 AU	1222.2 AU	2.86 %
7	0.59 Rf	20.8 AU	0.63 Rf	167.9 AU	10.09 %	0.67 Rf	0.4 AU	4919.4 AU	11.50 %
8	0.75 Rf	0.5 AU	0.77 Rf	13.4 AU	0.80 %	0.80 Rf	0.3 AU	283.3 AU	0.66 %
9	0.87 Rf	0.5 AU	0.91 Rf	38.6 AU	2.32 %	0.93 Rf	1.9 AU	838.6 AU	1.96 %

Table 1: Rf table for HPTLC Fingerprint of Madhuca longifolia

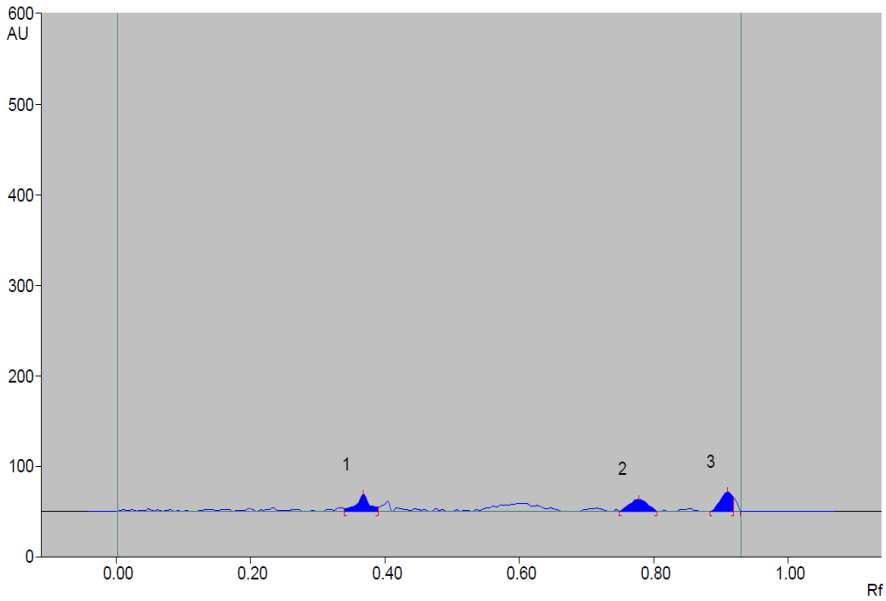

Fig2: HPTLC chromatogram of Curcuma aromatica rhizome extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.34 Rf	3.2 AU	0.37 Rf	19.1 AU	35.35 %	0.39 Rf	4.6 AU	298.0 AU	32.48 %
2	0.75 Rf	0.4 AU	0.78 Rf	13.4 AU	24.79 %	0.81 Rf	0.2 AU	304.0 AU	33.13 %
3	0.88 Rf	0.1 AU	0.91 Rf	21.5 AU	39.86 %	0.92 Rf	6.1 AU	315.5 AU	34.38 %

Table2: Rf table for HPTLC Fingerprint of Curcuma aromatica rhizome extract

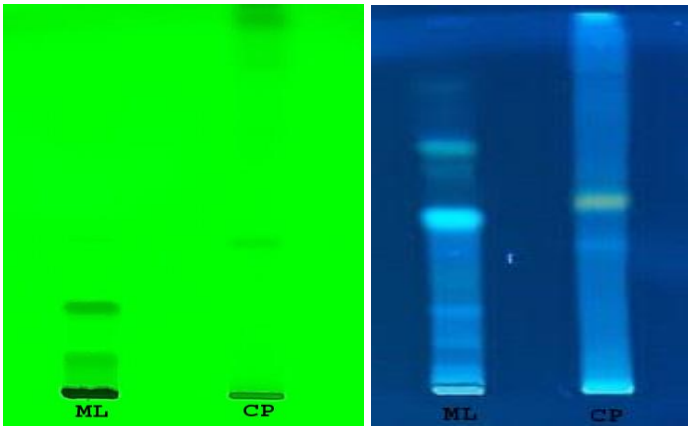

Fig 3: Photo documentation of HPTLC plates at 254 and 366 nm respectively.
(ML- *Madhuca longifolia;* CP- *Curcuma aromatica*)

Table 3 : Antibacterial activity of plant extracts

Sr. No.	Name of Bacteria	Zone of inhibition (mm)			
		Control D/w	M.longifolia	*C.aromatica*	*Standard drug*
1	*Staphylococcus aureus*	00 mm	03 mm	06 mm	28 mm
2	*Escherichia coli*	00 mm	02 mm	04 mm	24 mm
3	*Salmonella typhi*	00 mm	06 mm	03 mm	18 mm
4	*Bacillus subtilis*	00 mm	03 mm	06 mm	16 mm

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Staphylococcus aureus *Escherichia coli* *Salmonella typhi* *Bacillus subtilis*

Fig. 4 : Photographs of antibacterial activities.

Antibacterial activity:

It order to understand the growth inhibitory action of *M. longifolia* flower powder and *C. aromatica* rhizome powder the methanolic extracts were tested against pathogenic bacteria and results were obtained. The impact of plant extracts on bacterial growth measured in terms of zone of inhibition as noted in table no. 3. As mentioned in table methanolic flower extract of *M. longifolia* showed significant activity against *S.typhi* (33%) and followed by *B. subtilis* (18%), *S.aureus* (10%) and *E. coli* (8%). Similarly the antibacterial activity of rhizome extract of *C. aromatica* showed consideration activity against tested bacteria. It shows maximum activity against *Bacillus subtilis*(37%) followed by *S. aureus* (21%), *Salmonella typhi* and *Escherichia coli* (16%. It proves that both the plants have antibacterial potential.

Conflict of interest

The authors have no conflict of interest.

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