ISSN: 2279 - 0594

Available Online at www.jbpr.in

Journal of Biomedical and Pharmaceutical Research, Volume 3, Issue 4, 2014, 58-62

RESEARCH ARTICLE

MICROBIAL SENSITIVITY ASSAY & HPTLC ANALYSIS OF *TERMINALIA CATAPPA & ADATHODA VASICA*

Geetha Unnikrishnan[1*], Shweta V. Bhangale[2], Anju Unnithan[3], Sonali Patil[3]

[1*] Department of Zoology, Birla College of Arts, Science & Commerce, Kalyan- 421304, India

[2] Department of Bioanalytical sciences, Birla College of Arts, Science & Commerce, Kalyan- 421304, India

[3] Department of Biotechnology, Birla College of Arts, Science & Commerce, Kalyan- 421304, India

Received 22 July 2014; Accepted 4 August 2014

ABSTRACT

In the present study anti- bacterial acivity of *Terminalia catappa* & *Adathoda vasica* was evaluated to find the zone of inhibition and to set a HPTLC profile of these plant extracts to make out these plants as a source to formulate novel naturally derived antimicrobial formulations. Agar well diffusion assay of hydro- ethanol extract showed a dose dependent growth inhibitory effect on test organisms with maximum activity in *Terminalia catappa* extracts with an MIC in the range of 0.1 - 3 mg /ml. Whereas *Adhathoda vasica* showed moderate activity against test organisms. Phytochemical analysis explored the presence of different phytochemicals in the extract with maximum amount of saponins. The HPTLC fingerprinting studies showed significant fingerprint profile patterns in mobile phase Toluene: methanol (8:1, v/v/v) with maximum bands. The results suggest that *Terminalia catappa* leaf extracts could serve as a source of compounds to formulate herbal medicines against bacterial infections.

INTRODUCTION:

Natural plant products have long been used in control of microorganisms that insists a systematic study of medicinal plants in order to find active compounds. Exposure of plant bioactive compounds requires extraction from medicinal plants facilitating the pharmacology studies, leading to the synthesis of a more potent drug with reduced toxicity. The WHO has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards [1]

Terminalia catappa is widely grown in tropical regions of the world as an ornamental tree & *Adathoda vasica* is a perennial, evergreen and highly branched shrub (1.0 m to 2.5 mm height) with unpleasant smell and bitter taste [2]. *Terminalia catappa* belongs to the family combretaceae and *Adhatoda vasica* belongs to the family Acanthaceae. These plants are known for many ethnopharmacological activities [3] [4] [5] [6] [7]. Adhatoda has is included in the WHO manual "The Use of Traditional Medicine in Primary Health Care" which is intended for health workers in south-east Asia to keep them informed of the restorative utility of their surrounding flora [8]. But Leaves of these plants are involved in very few manufacturing processes. Thus considering their vast potentiality a systemic investigation was undertaken to screen the antibacterial activity & HPTLC fingerprinting of *Terminalia catappa* & *Adathoda vasica* to make out these plants as sources of antimicrobial compounds.

METHODS:

Preparation of Plant material:

Fresh leaves of *Terminalia catappa and Adhatoda vasica* were collected from the botanical garden of college campus. The plants were authenticated at Blatter's herbarium; St. Xavier's College, Mumbai and the specimens voucher were deposited in the St. Xavier's College The leaves were washed and dried in oven at a temperature of 60°C. The dried leaves were pulverized to a coarse powder in a mechanical grinder and passed through a sieve.

Solvent Extraction:

10gm of powder was suspended each in 100 ml of 75% ethanol for 72 hrs for 24hrs in a shaker. The extract was decanted, filtered with Whitman No. 1 filter paper and concentrated by evaporation.

Phytochemical screening:

Hydro-Ethanol extract was subjected to preliminary phytochemical screening using standard chemical tests [9] to determine the major chemical groups. Observations were made from two independent experiments.

Quantitative Estimation of Phytochemicals:

Quantitative analysis of the extract for total Phenol, Tannins, Saponins, Alkaloid and Flavanoid was carried out

*Corresponding author: Geetha Unnikrishnan |E-mail: unnikrishnangeeta@gmail.com

using standard procedures suggested by Folin-Ciocalteau, Folin- Denis, Obadoni- Ochuke, Horborne's Method and Bohm and Kocipai Abyazan respectively.

Evaluation of antimicrobial activity:

Preparation of inoculum:

Sterile Nutrient Broth was inoculated with bacterial isolates and incubated for 24hrs. Inoculum size was standardized by adjusting the optical density of the suspension to turbidity corresponding to spectrophotometric absorbance 0.5 at 540 nm.

Minimum Inhibitory Concentration (MIC) by agar well diffusion method:

The antimicrobial potential of extracts and fractions was evaluated according to their zone of inhibition against bacterial isolates. Minimum Inhibitory Concentration (MIC) is defined as the least concentration of the extracts that inhibit growth of organisms [10].

The hydro-ethanol extracts were diluted using two fold serial dilution method. 20 ml sterile nutrient agar medium was autoclaved and brought down the temperature around 50°C. 1ml of bacterial isolate suspension was added to each flask, mixed thoroughly and poured immediately on the plate [11]. Wells were made on the agar plate using a cork borer of 6mm and two fold serial dilutions of the extracts were added to the wells. After 24hrs of the incubation at 28°C the zone of inhibition was calculated using the formula: Zone of inhibition =Total Diameter – well diameter (6mm). The MIC values were taken as the lowest concentration range of the extracts that showed > 4mm diameter

HPTLC fingerprinting of extracts:

The HPTLC fingerprinting studies of *Terminalia catappa* and *Adhatoda vasica* was performed with many ratios of different mobile phase systems to obtain high resolution band patterns with minimized errors. Merck Silica gel 60 F_{254} TLC pre-coated plates were spotted with 10µl volume ethanol fraction using CAMAG Linomat 5 sample applicator. Separate track was maintained for each plant extract with separate peak development in different mobile phases. The compounds in the fraction were resolved in a CAMAG twin trough chamber saturated with different mobile phases and derivatized by spraying the plate methanolic sulphuric acid reagent(10%). The plates were scanned at 366nm before and after derivatization with CAMAG Scanner 4 equipped with winCATS Planar Chromatography manager software and photo documentation was done using CAMAG Reprostar.

RESULTS AND DISCUSSION:

Qualitative analysis:

The qualitative analysis of extract of *Terminala catappa* & Adhatoda vasica revealed the presence of a range of phytochemicals (Table 1)

Table 1: Phytochemical Screening of hydroethanol extract of *Terminalia catappa* and *Adhatoda vasica*

Phytochemicals	Terminalia catappa	Adhatoda vasica
Tannins	+	+
Saponins	+	+
Flavanoids	+	+
Alkaloids	+	+
Steroids	-	+
Phenols	+	+
Terpenoids	+	+
Cardiac Glycosides	+	+

Quantitative analysis:

Table 2 : Quantitative analysis of hydroethanol extract of *Terminalia catappa* and *Adhatoda vasica*

Phytochemicals	Quantitative analysis (%) per gram dry leaf powder	
	T. catappa	*A. vasica*
Tannins	12.43	5.33
Saponins	28.03	36.2
Flavonoids	10.7	8.9
Alkaloids	10	4.03

Antimicrobial screening:

A. vasica showed maximum inhibitory effect was seen on *Corynebacterium diphtheria* with the MIC in a range of 1.531-3.062 mg/ml. Whereas least effect was seen on *Bacillus licheniformis, Bacillus subtilis* with a range of 12.5-25mg/ml. *T. catappa*, showed maximum inhibitory effect on *Corynebacterium diphtheria* with the MIC in a range 0.191-0.382mg/ml and least effect was seen on *Klebsiella pneumonia* with a range of 1.531-3.062mg/ml.

Table 3: MIC of hydroethanol extract of *Adhatoda vasica* and *Terminalia catappa* against test organisms

Test organism	MIC range mg/ml	
	A.vasica	*T. catappa*
Escherichia coli	3.062-6.125	0.765-1.531
Klebsiella pneumonia	3.062-6.125	1.531-3.062
Pseudomonas aeruginosa	3.062-6.125	0.382-0.765
Corynebacterium diphtheria	1.531-3.062	0.191-0.382
Bacillus licheniformis	12.5-25	0.765-1.531
Bacillus subtilis	12.5-25	0.765-1.531

HPTLC Fingerprinting

The HPTLC fingerprinting studies of *Terminalia catappa* and *Adhatoda vasica* showed significant fingerprint profile patterns with Mobile phase ratios Toluene: methanol (8:1, v/v/v)(S1), Toluene: ethyl acetate: methanol (8:1:1, v/v/v) (S2), Toluene: ethyl acetate: methanol: glacial acetic acid (8:1:0.5:0.3, v/v/v/v)(S3). The finger print profile with S1 mobile phase system of ethanol extract of *Terminalia catappa* & *Adhatoda vasica* under 366 nm showed 15, 11 (Fig:1) peaks before derivatization and 15, 10 (Fig:2) peaks after derivatization respectively. Samples developed with S2 solvent system showed 11, 9 (Fig: 3) peaks before derivatization and 13, 9 (Fig: 4) peaks after derivatization under same conditions. Spots developed with S3 solvent system didn't show significant peaks before derivatization but showed 13, 12 (Fig: 5) peaks after derivatization.

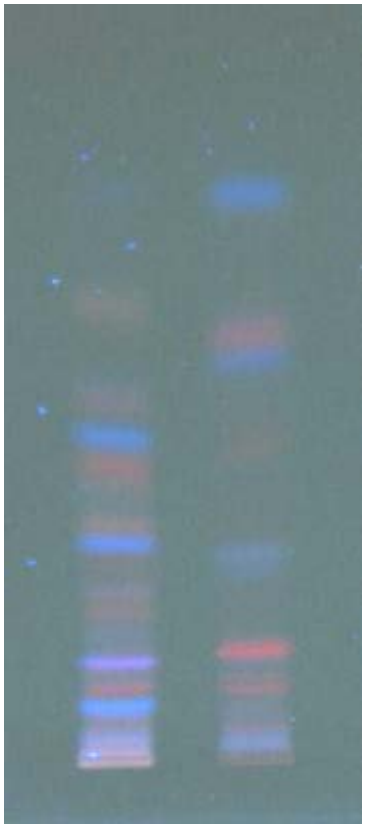

Figure 1: TLC with S1 mobile system before derivatization A *Terminalia catappa* B *Adhatoda vasica*

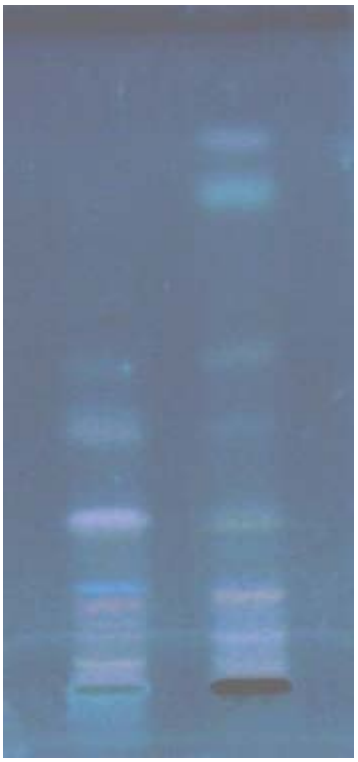

Figure 2 TLC with S1 mobile system after derivatization A *Terminalia catappa* B *Adhatoda vasica*

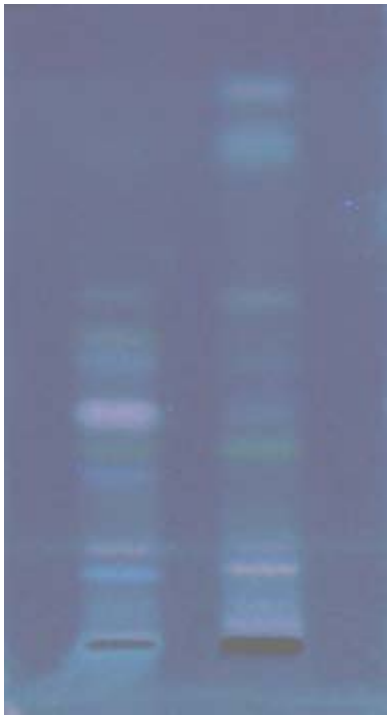

Figure 3: TLC with S2 mobile system before derivatization A *Terminalia catappa* B *Adhatoda vasica*

DISCUSSION:

In this present study, the ethanol extracts displayed a variable degree of antimicrobial activity against different test bacterial strains. The maximum activity of *Terminalia catappa* leaf extract is mainly due to the better solubility of the active compounds in ethanol solvent suggesting ethanol as a suitable solvent for the extraction of antimicrobial compounds from it. But in *Adhatoda vasica* ethanol solvent is not suitable for the extraction of antimicrobial compounds since it is showing moderate activity against test organisms. These findings lead to the support of De Boer et al [12] who suggested that the

activity of phytochemicals depends upon the solvents used for their extraction. . Both *T. catappa* and *A. vasica* showed maximum inhibitory effect on *Corynebacterium diphtheria*. The HPTLC analysis with Toluene: methanol 8:1 resolved maximum number of bands on TLC plate. Thus the same solvent can be used for further analysis for the detection and isolation of antimicrobial compound. As well the HPTLC fingerprint profiles along with their recorded Rf values, can serve as reference standard for further research on the medicinal properties of these plants. Such finger printing is useful in differentiating the species from the adulterant and act as biochemical markers for these medicinally important plants in the pharma. industry and plant systematic studies.

CONCLUSION:

The findings of the present study suggest the use of leaf extracts of *Terminalia catappa* as a source of compounds to formulate novel antibacterial herbal medicines.

CONFLICT-OF-INTERESTS:

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENTS:

The authors gratefully acknowledge the cooperation of colleagues from Birla College of Arts, Science and Commerce, India for carrying out the research.

REFERENCES:

1. WHO. Quality control method for medicinal plant material Geneva: WHO; (1998), 1-15.
2. Patel V. K., Venkata-Krishna-Bhatt. H., In vitro study of antimicrobial activity of *Adhatoda vasica* (L) (Leaf extract) on gingival inflammation. A preliminary report. Indian Journal of Medical Sciences (1984) 38 70-72.
3. Dhuley J. N., Antitussive effect of *Adhatoda vasica* extract on mechanical or chemical stimulation – induced coughing in animals. J. Ethnopharmacol., (1999) 67: 361-365.
4. Claeson U.P., Malmfors T., Wikman G., Bruhn J.G., *Adhatoda vasica*: A critical review of ethnopharmacological and toxicological data. J. Ethnopharmacol., (2000) 72: 1-20.
5. X. H. Tang., L. Gao., J. Gao et al., "Mechanisms of hepatoprotection of Terminalia *catapp*a L. extract on D-galactosamine-induced liver damage," American Journal of Chinese Medicine, (2004) 32,. 509–519.
6. S. C. Chu, S. F. Yang, S. J. Liu, W. H. Kuo, Y. Z. Chang, and Y. S. Hsieh, "In vitro and in vivo antimetastatic effects of Terminalia catappa L. leaves on lung cancer cells," Food and Chemical Toxicology, (2007), 45, 1194–1201.
7. Modak A.T., Rao M. R. R., Hypoglycemic activity of a non nitrogenous principle from the leaves of *Adhatoda vasica* Nees. Ind. J. Pharm., (1966) 28. 05-106.
8. WHO., The use of traditional medicine in primary health care. A manual for health workers in South-East Asia, SEARO Regional Health Papers, No. 19. New Delhi, (1990) 1-2.
9. Prashant Tiwari, Brimless Kumar, Mandeep Kaur, Gurpreet Kaur, Harleen Kaur, Phytochemical screening and Extraction: A Review. Internationale pharmaceutica sciencia. (2011) 1.
10. Odunayo R., Akinsulire., Ibukun E., Aibinu., Tayo Adenipekun., Toyin Adelowotan. Tolu Odugbemi, *In vitro* antimicrobial activity of crude extracts from plants *Bryophyllum pinnatum* and *kalanchoecrenata.*. Afr. J. Traditional, Complementary and Alternative Medicines. (2007) 4, 338 – 344.
11. G. Ramakrishnan, R. Kothai, B. Jaykar, T. Venkata Rathnakumar, *In vitro* Antibacterial Activity of different extracts of Leaves of *Coldenia procumbens*. International Journal of Pharm Tech Research. (2011). 1000-1004.
12. H J De Boer; A Kool; W R Mizirary; I Hedberg; J J Levenfors. J. Ethanopharmacol., (2005) 96, 461-469.

