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Preliminary Phytochemical Screening of Extract of the leaves of *Lantana camara* Linn against Uropathogen *E.coli*

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Key words : *Lantana camara,* Ethanolic extract, Uropathogens, Phytochemical screening, *E.coli.*

Lantana camara linn a flowering shrub is used in Ayurveda, Unani and Siddha medicines for treatment of various human diseases. Urinary tract infections are caused by bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae,* etc. Due to alarming incidence of antibiotic resistance in uropathogens herbal medicines have become more popular in the treatment of UTIs (Urinary tract infection) due to the belief that green medicine is safe, easily available and with fewer side effects. Ethanolic extracts of leaves of *Lantana camara* was tested against *E. coli,* a gram negative uropathogen isolated from clinically diagnosed UTI patients. The minimum inhibitory concentration (MIC) was carried out by broth macro dilution method. The ethanolic extract of the leaves of this plant was found to inhibit the in vitro growth of *E.coli*.The competent experimental results justify the use of this plant species as a better bactericidal agent.

INTRODUCTION

Lantana camara linn belonging to the family Verbenaceae is a notorious invasive weed that is toxic to grazing animals. It has negative impact on the ecosystem due to its luxurient growth (Sharma *et al*., 1988). The different parts of the plant have been used in the treatment of a wide variety of human diseases. A tea prepared from the leaves and flower was used against fever, influenza and stomach-ache. In Ghana infusion of the whole plant is used for bronchitis and the powdered root in milk is given to children for stomach-ache (Irvine 1961), the leaves extract of *Lantana camara* exhibit antimicrobial activity and contain verbascoside which possesses antimicrobial imunosuppressive and antitumor activities (Adiguzel et al 2005).

Six phenolic compounds in *L. camara* extract were identified by high-pressure liquid chromatography (HPLC): Salicyclic acid, jentisic acid, ?-resorcyclic acid, coconarin, ferulic acid and 6-MC cocomerin(Yi *et al*., 2006).

These secondry metabolites of plants serve as medicine in the treatment of various infectious disaeses. Previously, L.camara has been extensively investigated for the phytochemical compositions. Triterpenes (lantadane type), steroid and alkaloids are reported as major phytochemical consituents of *L. camara*. Phytochemical analysis of leaves extract of *Lantana camara* revealed presence of saponine, steroids, alkaloids, tannins, polyphenols, terpenoids and flavonoids as one of the chemical constituents. These secondry metabolites serve as medicine in the traetment of various infectious diseases . Secondary metabolites present in leaves of *L.camara* has not yet been studied sufficiently against gram negative uropathogens like *E. coli.* There is a constant need for new and effective therapeutic agents which are natural, stable, non toxic and multifunctional. There are very few studies and published information on antimicrobial activity of *L. camara* against uropathogen *E. coli.* Keeping all these things in consideration the leaf extract (ethanolic) of *L. camara* was choosen in the present study to screen antimicrobial activities against *E. coli.*

MATERIALS AND METHODS

1. **Collection of urine sample:** urine sample was collected from clinically diagnosed patients in sterile container by using clean-catch method. In this method mid-stream urine sample was collected. This reduces the risk of the sample being contaminated with bacteria from hands or the skin around the urethra and the tube that carries urine out of the body.

2. **Isolation and characterization of urine specimen:** for the isolation of Gram negative uropathogen E. coli urine samples were streaked on the nutrients agar plate and incubated at 37± 2°C for 24 hrs. After incubation, colonies were selected and identified on the basis of morphological and biochemical characters (Thomas 1995).

3. **Collection of plant samples:** The plant namely *Lantana camara* linn was collected from different areas of Gaya town.

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4. Processing of plant material: The leaves of *Lantana camara* linn were properly washed in tap water then rinsed in distilled water. The rinsed leaves were oven dried at a tempreture of 30-40°C for three days. The dried leaves were pulverized in a sterile electric blender, to obtain a fine powder and stored in airtight- glass containers, protected from sunlight until required for analysis.

5. **Extraction:** The ethanolic extract of leaves of *Lantana camara* linn was prepared by soaking 10g of dried powdered sample in 100 ml of ethanol for 24 hrs. The extract was collected by filtering through 5 layers of muslin cloth and concentrated at low tempreture. The prepared extract was preserved in a desicator for further study.

6. **Phytochemical investigation:** The ethanolic extract was subjected to qualitative phytochemical analysis for the different secondary plant metabolites (Brain and Turner, 1975; Sofowora, 1993 and Edeoga *et al*., 2005).

7. **Determination of Minimum Inhibition Concentration (MIC):** The MIC was carried out by broth macro dilution method (Brantner and Grein.,1994). The test organism was grown in nutrient broth medium to a concentration of 1×10^6 CFU/ml. For determination of MIC of leaves of *Lantana camara* linn the ethanoic extract of different fractions were prepared by serial dilution using distilled water to obtain concentration of 1mg/ml, 1.5mg/ml, 2mg/ml, 2.5mg/ml and 3mg/ml. Extract of about 0.5 ml (1mg-2.5mg/ml) was mixed with 4ml of nutrients broth inoculated with 0.5ml of bacterial suspension.

OBSERVATIONS

On the nutrients agar plate *E.coli* colonies showed large, thick, greyish white, mostly smooth and opaque colonies. On microscopic examinations done by Gram's staining. *E.coli* colonies were rod shaped structures. On biochemical examination E.coli fermented lactose resulted in the production of acid and gas. All the isolates have been observed to give positive result with indole test and all the isolates were found to give positive result with MR test but VP test showed negative result. None of the isolates utilized citrate.

Table-1

Cultural and Biochemical Characterization of E.coli

On Nutrients agar media	Large, white, moist smooth colonies
Gram's staining	Gram negative (-) rods
Lactose utilization	+(AG)
Catalase test	+
Indole test	+
MR-test	+
VP-test	–
Citrate utilization	–

(+) = positive (–) = Negative AG = Acid and Gas production

Table-2

Qualitative phytochemical screening of ethanolic leaf extract of *L. camara*

Sl. No.	Tested group	Leaf extract of *L. camara* linn (ethanolic)
1	Alkaloids	+
2	Saponins	+
3	Glycosides	+
4	Carbohydrates	+
5	Tannins	+
6	Flavonoids	+
7	Steroids	+
8	Triterpenoids	–

(+) = Positive/Presence (–) = Negative/Absence.

Table-3

Determination of the MIC of ethanolic leaf extract of *Lantana camara Linn*

against Gram negative uropathogens E.coli.

Bacterial pathogens	Bacterial growth at different concentration				
	1mg/ml	1.25mg/ml	2mg/ml	2.5mg/ml	3mg/ml
Escherichia coli	–	–	–	+	+

(–) = Growth, (+) = No growth.

Discussion: The inhibitory effects of leaves extract of *Lantana camara* linn has been showed in Table-3. The antibacterial activities were determined by measuring MIC. MIC determination clearly showed that ethanolic extract was effective on the tested Gram negative uropathogens *E. coli.* It is belived that crude extracts from medicinal plants are more biologically more active than isolated compounds due to their synergistic effects. Phytochemical screening revealed the presence of numerous secondry metabolites. These secondry metabolites of plants serve as medicine in the treatment of various infectious diseases. The MIC determination by broth macrodilution method is extensively used to investigate the antimicrobial activity of plant extracts for uropathogen *E. Coli.* MIC value exceeded 2mg/ml.

The MIC value for E.coli found to be 2.5 mg/m/. This tends to show that the active ingredients of the leaves are better extracted with ethanol. All of the identified compounds from plants are aromatic and saturated organic compounds.

Dsepite the avilability of antibiotics, UTI patients are usually prescribed herbal drug to prevent recurrent UTIs to sterilize urine because renal damage may occur in the presence of infection (Mangiarotti *et al*., 2000). The gram negative uropathogen evolved resistance against old and newly discovered drugs. This type of antibiotic resistance in uropathogen has led to screening of herbs and shrubs for their potential animicrobial activity. In the present investigation qualitative phytochemicals analysis of *L. camara* leaf ethanolic extract resulted in the presence of a number of phytochemicals like alkaloids, saponins, glycosides, carbohydrates, tannins, flavonoids and steroids. Saraf *et al*. (2011) also reported the presence of above phytochemicals . triterpenoids were found to be absent in the plant extract. But literature review of preliminary phytochemical screening of ethanolic extract of the leaves of *L. camara* showed the presence of terpenoids (Saikta *et al*., 2011). This may be due to geographical and climatic variations.

It is important to note that the use of ethanol as organic solvent concentrates more water soluble compounds including saponins and tannins. Saponin is the major component of plant that acts as antibacterial secondary metabolite (Sufferdini et al 2004). The mode of action of its antibacterial effects involves membraneytic properties (Aliyee *et al.,* 2012). Tannins are phenolic compounds have the ability to inactivate microbial adhesions, enzymes and cell envelop transport proteins (Cowan 1999).

In the current investigation choosen plant extract exhibited activity against uropathogen *E.coli*, the MIC of ethanolic extract of leaf was 2.5mg/ml. *L. camara* showed its antimicrobial potential against test pathogens which are involved in Urinary tract infections. Previous studies using extracts from lantana species showed that they were able to inhibit the growth of gram-positive bacterial strains (Junior Ajs *et al.,* 2005). However, in this study, the antimicrobial activity against gram negative (*E.coli*) bacteria was verified. In a study from India, it was reported that *L. camara* had remarkable antibacterial activity against *E.coli* (a MTcc drug- sensitive strain) (Udayprakash *et al*., 2011). The present sudy shows that the selected plant extract had sucessfully inhibited the selected gram negative urpoathogen *E. coli.* Ethanolic extract of leaf of *L. camara* showed significant value of MIC, a major source of medicine which is used for the treatment of UTIs. Traditionally people are using this plant on regular basis but scientific community not do accept this concept. Scientific evidence is needed for the purpose using this plant. The present result also provides evidence for medicinal use of our traditional knowledge.

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