IJPSR (2011), Vol. 2, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 May, 2011; received in revised form 21 May, 2011; accepted 25 July, 2011

UROPATHOGEN RESISTANT ESSENTIAL OILS OF *COLEUS AROMATICUS* AND *OCIMUM SANCTUM*

Rashmi Sahay Khare [1], Sourish Karmakar [2], Shanta Banerjee [3], Gopal Nath [4], Subir Kundu [2] and Kanika Kundu*[1]

Department of Chemistry, MMV, Banaras Hindu University [1], Varanasi, India
School of Biochemical Engineering, Institute of Technology, Banaras Hindu University [2], Varanasi, India
Plantachem GbR [3], Industrie- und Gewerbegebiet 21, 16278 Pinnow (UM), Germany
Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University [4], Varanasi, India

Keywords:

Coleus aromaticus, *Ocimum sanctum*, Essential oil, Carvacrol, Eugenol, Eugenol methyl ether, Urinary tract infection

Correspondence to Author:

Dr. Kanika Kundu

Senior Associate Professor, Chemistry Section, MMV, Banaras Hindu University, Varanasi – 221005, Uttar Pradesh, India

ABSTRACT

Urinary tract infections are common type of pathogenic infections in body. Essential oils of *Coleus aromaticus* and two varieties of *Ocimum sanctum* (Rama Tulasi and Shyama Tulasi) were compared for antibacterial activity against urinary tract infection (UTI) causing bacteria; *Staphylococcus aureus*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. *Ocimum sanctum* (Rama Tulasi) and *Coleus aromaticus* oils showed remarkable activity, later being more active. Least activity was observed in Shyama Tulasi variety of *Ocimum sanctum.* Constituents of the oils were analyzed by GC-MS and GC-FID. The essential oils have promising inhibitory effect with minimal inhibitory concentration (MIC) ranging from 0.5µl/ml -6µl/ml. The essential oils of two varieties of *Ocimum sanctum* have differences both in chemical composition and MIC values against UTI infection causing pathogens.

INTRODUCTION: Urinary tract infection (UTI) is the second most common type of infection in the body. It is reported that about 150 million people suffer from urinary tract infections per year worldwide [1]. UTI involves infection in the kidneys, ureters, and urethra caused by bacteria that may affect any part of urinary tract. In most cases bacteria travel to the urethra and multiply causing kidney infection [2]. UTI is more common in women as compared to men. It is reported that about 35% of healthy women suffer symptoms of Urinary tract infection and about 5% of women each year suffer with the problem of painful urination (dysuria) and frequency [3].

Several potent antibiotics are available for the treatment of UTI, but increasing drug resistance among bacteria has made therapy of UTI difficult. Bacteria have the genetic ability to transmit and acquire resistance to drugs [4]. Plant metabolites are great source of bioactive compounds and serve as better alternative to combat with the diseases caused by multidrug resistant organisms. Since plant secondary metabolites are natural isolates, they are safe to use as therapeutic agent. In India plant parts and their extracts are in use from very ancient time.

WHO reported that majority of Indian population depends on plants and their extracts for primary health care [5]. A number of studies have been conducted on different plant parts to prove their antimicrobial efficacy [6, 7, 8, 9, 10]. Plant extracts are mixture of many minor and major compounds of different nature therefore the chance of bacterial resistance is minimized.

Also the synergistic effect of the mixture of phytochemicals play important role to use plant extracts as antimicrobial agents [11]. Organic solvent fractions of *Ocimum sanctum* has been reported to have activity against UTI infections [12, 13]. Fresh leaves of *Coleus aromaticus* were also found to be active against UTI causing bacteria [14]. Present work focuses on antibacterial activity of essential oils of *Coleus aromaticus* and *Ocimum sanctum* on UTI causing microorganisms *Staphylococcus aureus*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis.* The work also focuses on the comparison of the activity of two varieties of *Ocimum sanctum* (Rama Tulasi and Shyama Tulasi) grown locally. The constituents of the oils were analysed by GC-MS method.

MATERIALS AND METHODS:

Plant material: Small plants and plant parts of two varieties of *Ocimum sanctum* and *Coleus aromaticus* were grown in the month of April 2009, in small plot area of garden of MMV, Banaras Hindu University, Varanasi, India. Middle aged leaves of plants were harvested for essential oil extraction in month of September - October, 2009. Two verities of *Ocimum sanctum*, Rama Tulasi with light green leaves, while Shyama Tulasi with deep purple colour leaves are commonly found in North India.

Essential oils were extracted by hydro- distillation method with help of Clevenger's apparatus. Fresh leaves (250 g) of each plant were finely chopped, put in 5 L round bottom flask and filled with 2 L doubled distilled water. The floated essential oils were collected after draining water from Clevenger's apparatus. Essential oils were dried over anhydrous Sodium sulfate and collected in micro-tubes. They were stored in refrigerator at 4^{o}C till further analysis.

Analysis of the essential oils: Oil samples were analyzed by Gas Chromatography, HP 5890 Ser. III, autosampler HP 7636 with detection FID, electronic integration system. Optima 625 column was used, 1.8 µm (60 m X 0.32 mm) with Nitrogen gas as a carrier. Temperature program was as follow: Initial temperature 40°C held for 3 minutes, increased up to 230°C at the rate of 3°C/minutes, increased to final temperature 250°C at the rate of 20°C/minutes and held at the final temperature for 10 minutes. Analysis time was 43.3 minutes. Samples (1 µl) were injected for analysis. GC-MS analysis was done on HP 5971 using the same column and temperature program but with helium gas as a carrier. The ion source temperature and ion inlet temperature were 200^{o}C and 210^{o}C respectively. The ionization voltage and detector voltage was 70 eV (electron Volt) and 1.5 Volts respectively. Compound identification was done on the basis of available GC-MS data library.

Antimicrobial Activity Screening: The antimicrobial activity of essential oils was tested against Gram negative and Grams positive bacteria by disc diffusion method and agar dilution method. The preliminary screenings of essential oils were checked through paper disc diffusion method on Muller- Hinton agar (MHA) plates. Firstly all the strains *Staphylococcus aureus*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumoniae*, *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* were cultured on Muller- Hinton agar plates at 37^{o}C.

These clinical strains were obtained from Institute of Medical Science, Banaras Hindu University, Varanasi, India. After that the density of fresh culture of each strain was prepared to McFarland Standard 1 in saline water and with help of cotton swab were inoculated all over the surface of Muller- Hinton agar plates. Subsequently, sterilized filter paper (Whatmann filter paper no. 1) discs (diameter 6mm) were saturated with different volumes of three types of essential oils were put on agar plates. The plates were incubated at 37°C for 18 h for the observation of inhibition zone. Essential oils showing antimicrobial activities were later tested for minimal inhibitory concentration (MIC) against each bacterial culture.

Minimal Inhibitory Concentration (MIC) Assay: MIC estimation was carried out with a little modification [5]. 20 ml Mueller-Hinton agar with tween- 20 were autoclaved and solidified at 40 - 45^{0}C. Different volumes of essential oils were added in flask, mixed thoroughly and oil containing agar media was immediately poured on previously autoclaved Petri plates. The concentrations of essential oils in different plates were 0.5 µl/ml, 1 µl/ml, 2 µl/ml, 3 µl/ml, 4 µl/ml, 5 µl/ml and 6 µl/ml.

To maintain the same inoculums density for activity test, McFarland standard 1 according to National Committee for Clinical Laboratory Standards was prepared [15]. After solidification of agar plates, with help of sterilized swab bacterial suspension were spotted on plates. One plate contains 16 bacterial strains. Inoculated plates were allowed to dry up a little and then incubated at 37°C for overnight. After 24 hours, essential oils containing plates were compared with control plate containing sterile saline (1%) solution. The minimum inhibitory concentration was evaluated by observing for the absence of visible growth of each bacterium. The experiment was performed three times for consistency studies.

RESULTS AND DISCUSSION: All of the three oils were found to be transparent yellow liquid, with strong aromatic smell. Yields and density of three types of essential oils had significant difference. Percentage yields (w/v) were 0.37, 0.30, and 0.24 whereas the densities were 1.14 g/ml, 1.10 g/ml and 1.21 g/ml for Rama Tulasi, Shyama Tulasi and *Coleus aromaticus* respectively.

Figure 1(a) shows GC-MS chromatogram of *Coleus aromaticus* essential oil. In this chromatogram peak 1 is identified as α-pinene, peak 2 is identified as linalool, peak 3 is identified as 4-terpineol, peak 4 is identified as carvacrol, peak 5 is identified as eugenol, peak 6 is identified as β-caryophyllene and peak 7 is identified as α- caryophyllene. **Figure 1(b)** shows GC-MS of Shyama Tulasi. In this chromatogram peak 2 shows major peak with component eugenol methyl ether. Other peaks 1 and 3 shows presence of eugenol and β-caryophyllene respectively. **Figure 1(c)** represents GC-MS chromatogram of Rama Tulasi. In which peak 1 major peak shows the presence of eugenol, peak 2 is eugenol methyl ether and peak 3 is β-caryophyllene.

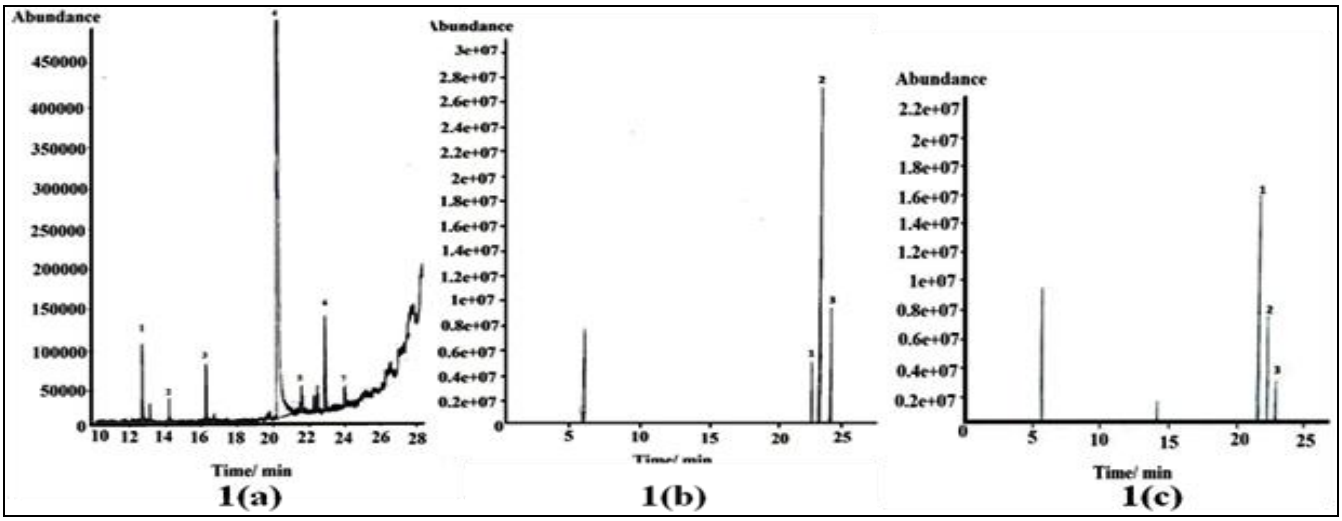
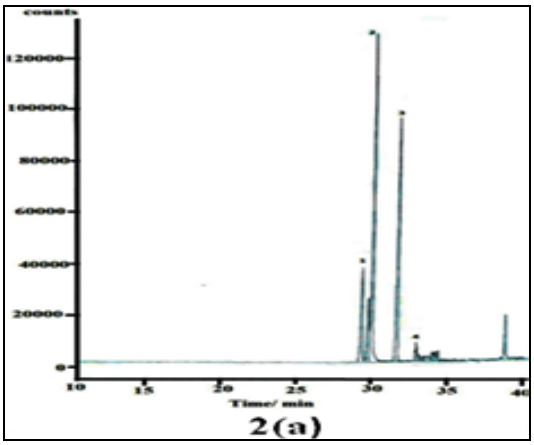


FIGURE 1: GC-MS SPECTRA FOR THREE TYPES OF ESSENTIAL OILS: 1(A) *COLEUS AROMATICUS;* 1(B); *OCIMUM SANCTUM* (SHYAMA TULASI) 1(C) *OCIMUM SANCTUM*; (RAMA TULASI)

Thus, there is clear difference in major compound of two varieties of *Ocimum sanctum*. Rama Tulasi has eugenol as a major compound where as Shyama Tulasi contains methyl eugenol as a major component. **Figure 2(a)** shows GC-FID of Shyama Tulasi in which peak 1 is eugenol (15.41%), peak 2 is eugenol methyl ether (39.78%), peak 3 is β-caryophyllene (22.96%) and peak 4 is α-caryophyllene (1.54%). **Figure 2(b)** shows GC-FID of Rama Tulasi in which peak 2 (Z) - methylcinnamate (3.18%), peak 3 is eugenol (22.96%), peak 4 is eugenol methyl ether (4.31%), peak 5 is (E)-methylcinnamate (16.00%), peak 6 is β-caryophyllene (15.90%) and peak 7 is α-caryophyllene (1.37%). Peak 1 is of an unknown compound but is present in significant amount.



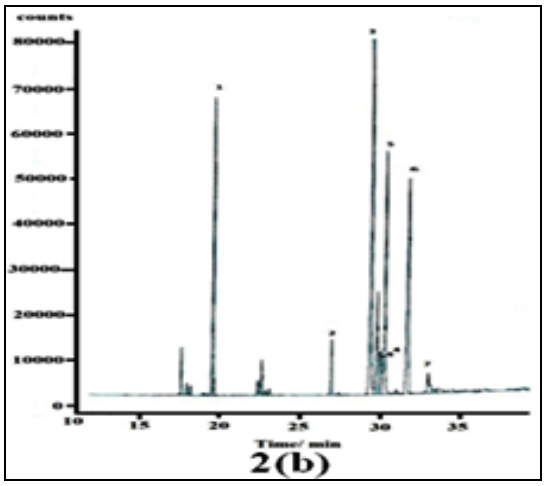


FIGURE 2: GC-FID SPECTRA FOR COMPARATIVE STUDY OF TWO VARIETIES OF *OCIMUM SANCTUM*: 2(A) SHYAMA TULASI; 2(B) RAMA TULASI

Comparative MIC of different essential oils: Antibacterial activity of the three essential oils was studied on the test organisms known to cause UTI. Essential oils of *Coleus aromaticus* and Rama Tulasi variety of *Ocimum sanctum* showed promising activity on UTI causing bacteria (**Table 1**). Best inhibitory activity was observed in case of *Klebsiella oxytoca* and *Proteus mirabilis* with *Coleus aromaticus* oil, MIC being 0.5 µl/ml. Low MIC values with *Coleus aromaticus* oil as compared to the two varieties of *Ocimum sanctum* clearly indicates its greater potentiality to treat UTI.

Comparative study of two verities of *Ocimum sanctum*: Inhibitory activity of the two varieties of *Ocimum sanctum* oils against *Klebsiella oxytoca, Proteus vulgaris, E. coli* and *Klebsiella pneumoniae* were significantly different (Table 1). GC-MS and GC-FID analysis results (**Table 2**) clearly show remarkable qualitative and quantitative difference in their chemical composition. Rama Tulasi contains eugenol (22.98%) as the major constituent of the oil, followed by (E)-methylcinnamate (16.00%) and β- caryophyllene (15.90%). Shyama Tulasi, on the other hand, contains eugenol methyl ether as the major component (39.78%) of the oil. Concentration of eugenol was less (15.41%) whereas (E)-and Z-methyl cinnamate were completely absent.

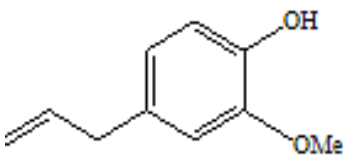
TABLE 1: COMPARATIVE MINIMAL INHIBITORY CONCENTRATION (MIC) OF THREE TYPES OF ESSENTIAL OILS

sBacterial strain	*Ocimum sanctum* (Shyama Tulasi)	*Ocimum sanctum* (Rama Tulasi)	*Coleus aromaticus*
Staphylococcus aureus	**~ 2 µl/ml**	**~ 2 µl/ml**	**~ 1 µl/ml**
Klebsiella oxytoca	**~ 2 µl/ml**	**~ 1 µl/ml**	**~ 0.5 µl/ml**
Proteus vulgaris	**~ 6 µl/ml**	**~ 5 µl/ml**	**~ 4 µl/ml**
E. coli	**~ 4 µl/ml**	**~ 3 µl/ml**	**~ 2 µl/ml**
Klebsiella pneumoniae	**resistant**	**~ 5 µl/ml**	**~ 3 µl/ml**
Pseudomonas aeruginosa	**5 µl/ml**	**~ 5 µl/ml**	**~ 3 µl/ml**
Proteus mirabilis	**~ 1 µl/ml**	**~ 1 µl/ml**	**~ 0.5 µl/ml**

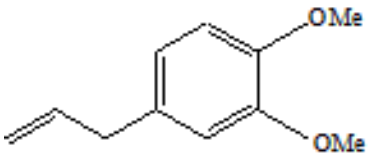
TABLE 2: GC-FID RESULTS FOR COMPARATIVE STUDY OF TWO VARIETIES OF *OCIMUM SANCTUM*

Compound	Rama Tulasi	Shyama Tulasi	Retention Time (min)
(Z)-Methylcinnamate	3.18	0.00	26.84
Eugenol	22.98	15 .41	29.26
Eugenol methyl ether	4.31	39.78	29.92
(E)-Methylcinnamate	16.00	0.00	30.14
β-Caryophyllene	15.90	22.96	31.57
α-Caryophyllene	1.37	1.54	32.89

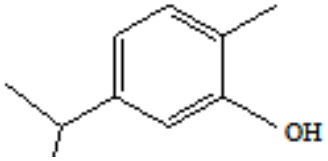
Different groups of worker have studied antibacterial activity of plant volatile oils on different organisms [16]. In most of the cases, the presence of free phenolic -OH group in compounds (**Figure 3**), as in eugenol and carvacrol, gives better inhibitory action in comparison to alkylated analogues.



EUGENOL



EUGENOL METHYL ETHER



CARVACROL

FIG. 3: STRUCTURE OF MAJOR COMPOUNDS PRESENT IN ESSENTIAL OILS

Our results further support the reported results [16]. Carvacrol and eugenol containing essential oils are more effective against bacteria as compared to eugenol methyl ether as they have free oxygen moiety. It is well understood by more activity of carvacrol containing oil (*C. aromaticus*) compared to eugenol and eugenol methyl ether containing essential oils (*Ocimum sanctum*).

CONCLUSION: From the study we conclude that essential oils of the three plants of Lamiaceae family showed antimicrobial activity against UTI infection. Difference in antibacterial activities is due to presence of different type of phenolic compounds as a major constituent of oils. Thus folk medicinal plants can be as effective as modern drugs to combat urinary tract infections.

REFERENCES

1. Stamm WE and Norrby SR: Urinary tract infections: disease panorama and challenges. J. Infect. Dis. 2001; 183:s1-s4.
2. Bethesda MD: National kidney and urologic diseases information clearing house. NIH Publication 2005; 08:3925.
3. Hootan TM: Urinary tract infection in adults,In: Johnson R.J., Feehally J., (Eds). Comprehensive clinical nephrology, 2nd ed, London: Mosby, 2003; 731:744.
4. Soulsby J: Resistance to antimicrobials in humans and animals. Braz. J. Med. 2005; 331:1219-1220.
5. Prabuseenivasan S, Jayakumar M and Ignacimuthu S: In vitro antibacterial activity of some plant essential oils. BMC Complementary and Alternative Medicine 2006; 6.
6. Cohen ML: Epidemiology of drug resistance: implications for a post-antimicrobial era. Science 1992; 257:1050-1055.
7. Almagboul AZ, Bashir AK, Farouk A and Salih AKM: Antimicrobial activity of certain Sudanese plants used in folkloric medicine. Screening for antibacterial activity. Fitoterapia1985; 56:331-337.
8. Izzo A, Di Carlo G, Biscardi D, Fusco R, Mascolo N, Borreli F, Capasso F, Fasulo MP and Autore G: Biological screening of Italian medicinal plants for antibacterial activity. Phytother. Res., 1995; 9:281-286.
9. Mandal P, Sinha Babu SP and Mandal NC: Antimicrobial activity of saponins from Acacia auriculiformis. Fitoterapia 2005; 76:462-465.
10. Srinivasan D, Sangeetha NS and Lakshmanaperumalsamy P: Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharmaco 2001; l74:217-220.
11. Pauli A: Amicbase– Essential Oils Supplementary Information. ReviewScience, 90513 Zirndorf, Further Str. 13.
12. Sharma A, Chandraker S, Patel VK and Ramteke P: Antibacterial Activity of Medicinal Plants against Pathogens causing Complicated Urinary Tract Infections. Indian J Pharm Sci. 2009; 71: 136–139.
13. Jain R, Kosta S and Tiwari A: Ayurveda and Urinary Tract Infections, Young Pharm 2010; 2: 337.
    Pritima RA, Selvaraj R and Pandian RS: Antimicrobial activity of Coleus aromaticus (Benth) against microbes of reproductive tract infections among women Afr. J. Infect. Diseases 2008; 1: 18 – 24.
14. NCCLS (National Committee for Clinical Laboratory Standards) *Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically*. In Approved Standard M100 S12 Wayne. PA, NCCLS, 2002.
15. Dorman HJD and Deans SG: Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. of Applied Microbio. 2000; 88:308–316.

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