# Bioefficacy of *Heliotropium ellipticum* Ledeb.I. Antimicrobial Screening

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**Aerial parts of *Heliotropium ellipticum* were sequentially extracted in petroleum ether, benzene, chloroform, ethanol and alkaloid rich fractions (petroleum ether and chloroform). Various extracts and reference antibiotics (tetracycline/mycostatin) were screened against selected bacteria and fungi and the resultant inhibition zone(s) and the activity index of crude drug were measured. The sequential extracts exhibited weak antimicrobial activity but it is interesting that the alkaloid-rich fractions demonstrated pronounced activity.**

HELIOTROPIUM species have been widely investigated for chemical constituents such as alkaloids, steroids, flavonoids and for bioactivities[1,2]. Except our earlier reports[3] on the isolation and identification of alkaloids there have been no reports in the literature on the phytochemistry or pharmacological activities in *H.ellipticum*, which is a wild plant of arid zone of Rajasthan. Hence, the present work was carried out with an aim to identify antimicrobial agent(s) from this plant species.

*Heliotropium ellipticum* Ledeb. (Syn. *H.eichwaldi* Steud.; family: Boraginaceae) collected (April - May,1997). From Agriculture Research Station, Durgapura, Jaipur and authenticated from the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India was used in the present investigation. Shade-dried powdered plant material (500 g) was Soxhlet extracted (24 h) in petroleum ether (60-80°, benzene, chloroform and ethanol in succession. Each of the extract was concentrated and dried under pressure. Similarly, a portion of the ethanolic concentrate (4 g) was treated[4] with 5% $H_2SO_4$ and later, it was filtered, basified with $NH_4OH$ (pH 10) and sequentially fractionated with pet. ether (Fr.1), chloroform (Fr.2) and diethyl ether (Fr.3). Each of the fractions was concentrated and examined on thin layer chromatography (silica gel G;solvent:chloroform-methanol-ammonia, 85 : 14:1; detector-Dragendorff's reagent)[5] which revealed four orange-red spots (A-D) in pet. ether and seven spots (E-K) in chloroform fraction. Based on co-tlc (colour reactions and $R_f$) and other spectral studies, spot H-K could be identified as europine, heliotridine, lasiocarpine and lasiocarpine-N-oxide respectively. However, the remaining ones could not be identified due to their poor yields.

Pure cultures of bacteria, *Escherichia coli, Klebsiella pneumoniae* and *Bacillus thuringiensis* (obtained from S.M.S. Medical College, Jaipur) and fungi, *Aspergillus niger, Rhizoctonia phaseoli* and *Penicillium crysogenum* (from Seed Pathology Laboratory, Department of Botany, University of Rajasthan, Jaipur) were used. The selected bacteria were grown on Nutrient Broth medium and incubated at 37° for 48 h,and maintained by transferring to fresh medium every 48 h. However, fungi were grown on Potato Dextrose Agar (PDA) medium by incubating at 27° for 48 h and maintained by periodic subculturing to fresh medium.

For antimicrobial assy, filter paper disc (6 mm) diffusion method[6] was adopted using test extracts (4 mg/disc), the isolated compound (2 mg/disc) and the reference drugs (tetracycline - 1 mg/disc and mycostatin - 1 mg/disc) were used.

It is interesting that out of sequential extracts, petroleum ether fraction failed to demonstrate any activity as against trace activities of other extracts. Likewise, among the alkaloid-rich fractions, eleven Dragendorff's positive compounds were located out of which unknown A-C, F and K did not exhibit any activity. However, compound E,G

**Table 1 : Antimicrobial Activity of Extracts of *Heliotropium ellipticum* Ledeb**

| Test Microorganisms | | I Sequential | | | | II Alkaloid-rich | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PE | $C_6H_6$ | $CHCl_3$ | EtOH | Pet ether | | | $CHCl_3$ | | | | | | | |
| | | | | | | A | B | C | D | E | F | G | H | I | J | K |
| **I. BACTERIA** | | | | | | | | | | | | | | | | |
| *E. coli* | IZ[a] | - | - | - | - | - | - | - | 2.42 | 9.85 | - | 7.95 | 6.59 | 6.32 | 8.42 | - |
| | AI[b] | | | | | | | | 0.10 | 0.38 | - | 0.29 | 0.22 | 0.26 | 0.35 | |
| *B. thuringiensis* | IZ | - | - | + | + | - | - | - | - | 5.38 | - | 4.42 | 7.94 | 5.95 | 4.48 | - |
| | AI | | | | | | | | | 0.20 | - | 0.17 | 0.28 | 0.22 | 0.17 | |
| *K. pneumoniae* | IZ | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | AI | | | | | | | | | | | | | | | |
| **II. FUNGI** | | | | | | | | | | | | | | | | |
| *A. niger* | IZ | - | - | + | - | - | - | - | 2.12 | 3.23 | - | 2.12 | - | - | 1.28 | - |
| | AI | | | | | | | | 0.32 | 0.60 | | 0.47 | | | 0.28 | |
| *R. phascoli* | IZ | - | + | - | - | - | - | - | - | 7.85 | - | 2.38 | - | - | 4.58 | - |
| | AI | | | | | | | | | 1.58 | | 0.70 | | | 1.19 | |
| *P. crysogenum* | IZ | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - |

All the values are expressed as mean of three relicates.

[a]Inhibition zone (IZ in mm) includes the diameter of disc (6 mm).

[b]Activity Index (AI) is calculated as : Inhibition area of sample/inhibition area of standard.

(+) - Trace activity

(-) - Not measurable

Abbreviations used : PE = Petroleum ether; $C_6H_6$= Benzene; $CHCl_3$ = Chloroform; EtOH = Ethanol, Alkaloids : Unknown A-G; H = Europine; I = Heliotridine; J = Lasiocarpine; K = Lasiocarpine-N-oxide.

and J were found to be active against all the test bacteria and fungi , except *K. pneumoniae* and *P. crysogenum*. It is noteworthy that among the identified alkaloids, compounds H-J exhibited substantial activity against the bacteria as compared to fungi, except of lasocarpine-N-oxide which exhibited no activity. It is concluded that the alkaloids isolated from chloroform fraction has increased activity as against petroleum ether.


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# Microwave Assisted Synthesis of New Bioactive 1, 3,4-Thiadiazolyl Substituted 1, 3,4-Oxadiazoles


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**A series of new 2- [5'- methyl-1, 3,4-thiadiazolyl] -5-aryl-1,3,4-oxadiazoles have been synthesised by the reaction of 5-methyl-1, 3,4-thiadiazolyl-2-thioacetic acid hydrazide with appropriate aromatic acids in $SoCl^2$ under microwave irradiation in open vessels using a domestic microwave oven as compared to the conventional method. The reaction rate has been improved tremendously. These oxadiazoles have shown promising antifungal activity against *A. niger* and *A. Flavous***


1 3, 4-Oxadiazoles have been extensively investigated by the organic chemists due to their close association with various types of biological activities[1-3]. In addition, 1,3, 4-thiadiazoles are potent antibacterial antifungal and antiviral agents[4-6]. Recently, accelerating the rate of a wide range of chemical reactions using microwave dielectric heating technique has become a field of wide interest[7-10].

In view of the importance of 1,3,4-oxadiazoles as potential pharmacological agents, substantial reduction in reaction time under microwave irradiation is of interest to us to prepare the title compounds starting from 2-mercapto-5-methyl-1,3,4-thiadiazole which is a side chain at c-3 position of an antibiotic drug cefazolin sodium[11].

Melting points were taken on Thomas Hoover apparatus and are uncorrected. The IR spectra ($\upsilon_{max}$ in $cm^{-1}$) of the synthesised compounds were recorded on 1710 Perkin Elmer FTIR Spectrophotometer using KBr discs and $^1H$ NMR on FT NMR Hitachi R-600 using TMS as internal reference (Chemical Shifts in δppm). 2-Mercapto-5-methyl-1, 3,4-thiadiazole (1) was purchased from Aldrich Chemical Co.