



Research Journal of
**Medicinal
Plant**

ISSN 1819-3455



Academic
Journals Inc.

www.academicjournals.com

Antibacterial Activity of Isolated Constituents and Extract of Roots of *Inula racemosa*

¹P.D. Lokhande, ¹K.R. Gawai, ¹K.M. Kodam, ²B.S. Kuchekar,
¹A.R. Chabukswar and ¹S.C. Jagdale

¹Department of Chemistry, Center for Advanced Studies, University of Pune, Pune, India

²Department of Pharmaceutical Chemistry, Government College of Pharmacy,
Karad, India 415124

Abstract: The resistance of different bacteria to the current antibacterial agents, toxicity of the antibacterial agents and the cost of the treatment has led to the development of new active molecules against the bacteria. Since ancient times medicinal plants have been used for the treatment of bacterial infections. The roots of the plant *Inula racemosa* has been used as folk medicine in East Asia and Europe. However, no systematic data is available on the antibacterial activity profile of the different constituents of *Inula racemosa*. In the present studies, attempt has been made for isolation of root constituents of *Inula racemosa* (Compositae) and evaluation of its antibacterial activity. The constituents were isolated and purified by column chromatography. The structure of the isolated constituents were confirmed by spectral analysis and were used for the determination of the antibacterial activity of *Inula racemosa* against various microorganisms. The constituent alantolactone showed maximum antibacterial activity as compared to other constituents and ethyl acetate extract of the roots.

Key words: Alantolactone, antibacterial activity, MIC

INTRODUCTION

Bacterial infections have posed a great challenge in front of us. The emergence of bacterial resistance to the currently available antibacterial agents necessitates the further research in the discovery of new safe and effective antibacterial agents (Davis, 1994). The medicinal plants have shown a promising alternative for the treatment of infectious diseases. In the antibacterial research, the vast majority, 78%, of the new chemical entities are natural or natural product-derived molecules (Newman *et al.*, 2003). *Inula racemosa* (Compositae) has been used as traditional medicine in East Asia and Europe (Okuda, 1986). In China, *Inula racemosa* has been prescribed for abdominal pain, acute enteritis and bacillary dysentery (Tsewang, 1994). The roots are widely used as indigenous medicine, as an expectorant and in veterinary medicine as a tonic (Chopra *et al.*, 1956). Native Americans used this plant for treatment of tuberculosis (Moerman, 1986). *Inula racemosa* blocks the adrenaline induced hyperglycemia, medicinal uses of plant are well documented in the literature (Tripathi and Chaturvedi, 1995). *Inula racemosa* can be prescribed in combination with guggul (*Commiphora Mukul*) for curing myocardial ischemia (Singh *et al.*, 1993). The onset of ischemia was significantly delayed when the rabbits were pretreated with *Inula racemosa* and severity was less pronounced as compared to controls (Dwivedi and Somani, 1988).

Phytochemical investigation of the plant showed the presence of alantolactone, isoalantolactone, dihydroalantolactone, dihydroisoalantolactone, sitosterol, daucosterol, inulinolide, aploxene, phenylacetonitrile and isoinunal (Wang *et al.*, 2000).

Corresponding Author: P.D. Lokhande, Department of Chemistry, Center for Advanced Studies, University of Pune, Pune, India 411007 Tel: 91-20-25601225/535 Fax: +91-20-25691728

Some of the sesquiterpene lactones of *Inula racemosa* has the pharmacological activities (Tripathi *et al.*, 1988). Alantolactone and isoalantolactone are the major constituent of *Inula racemosa*, they possess anti-fungal and antihelmintic activities (Satyawati *et al.*, 1987). The plant thus has a better pharmacological activity profile, therefore systematic investigation of the antibacterial activity of the plant may lead to the development of novel antibacterial agents. Use of currently available antibacterial agents is limited for many reasons such as poor solubility, low potency, emergence of resistant strains and toxicity. Available literature indicates that the constituents of *Inula racemosa* and the plant extract have not been studied systematically for their antibacterial activities.

Hence, the aim of the present study was to isolate and study the antibacterial activity of the different constituents and ethyl acetate extract of the plant *Inula racemosa*.

MATERIALS AND METHODS

Plant Material

The dried roots of the *Inula racemosa* were obtained from local market of Pune, India. The plant material was authenticated from Botanical Survey of India, Pune, India.

Chemicals

n-Hexane, ethyl acetate, methanol, chloroform, benzene, toluene and dimethyl formamide were purchased from Merck India Ltd., Mumbai, India. Nutrient agar was purchased from Hi-media, Mumbai, India. Streptomycin (Nicholas, India) was purchased from the local market of Pune, India. Silica gel chromatographic grade for the separation was obtained from Rankem, India. All other chemicals used were of high purity.

Bacterial Strains

Escherichia coli, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Serratia marcescens*, *Shigella dysenteriae* and *Staphylococcus aureus* were obtained from the Microbiology Department, University of Pune, Pune, India. All the strains were maintained on nutrient agar medium.

General Experimental Procedure

Melting points were determined in a open capillary (Gallenkamp melting point apparatus) and were uncorrected. IR spectra was recorded on Shimadzu 8400 FT infrared spectrometer. ¹H NMR was recorded on Varian-Mercury (300 MHz) FT NMR spectrophotometer with chemical shift data reported in ppm. Mass spectra were recorded on GC-MS Shimadzu (QP 5050) Mass spectrophotometer.

Extraction and Isolation

The dried and hard roots were first chopped into small pieces and crushed in mortar pestle. This crushed material was further grinded in blender to make a fine powder. The root powder was soaked in different solvents, like n-hexane, ethyl acetate, methanol, chloroform, benzene, toluene, etc. and subjected to Thin Layer Chromatographic (TLC) analysis in various solvents. The TLC plates were observed under UV as well as in iodine chamber. Ethyl acetate extract has shown the presence of maximum spots on TLC plate and hence has been selected for the extraction of the root constituents of the plant.

The dried roots (200 g) of *Inula racemosa* were extracted with ethyl acetate at room temperature for 48 h. This extract was then filtered and evaporated under reduced pressure to obtain a viscous mass (6 g). Some quantity of ethyl acetate extract was preserved for antibacterial activity and remaining

extract was chromatographed over silica gel using hexane as eluent (Tan *et al.*, 1998). A total of twenty fractions were (50 mL each) collected. Major constituents were identified by using spectral techniques like IR, NMR and GC-MS spectroscopy. The extraction of the plant resulted in the isolation of three known constituents. The isolated constituents were then subjected to the evaluation of their antibacterial activity.

Antibacterial Activity

Antibacterial activity of the isolated constituents and the crude extract was determined by the well diffusion method (Rios *et al.*, 1988; Mosquera *et al.*, 2004). The test constituents and the ethyl acetate extract were dissolved in dimethyl formamide (DMF). The microbial cultures were grown at 37°C for 24 h and then approximately diluted by 0.9% w/v sterile saline solution to obtain a cell suspension of 10^5 cfu mL⁻¹. Diluted inoculum (0.2 mL⁻¹, 10^5 cfu mL⁻¹) of test microorganisms were spread on nutrient agar plates. Wells of 6 mm diameter were punched into the agar medium and filled with 20 µL each of three isolated constituents, ethyl acetate extract at a concentration of 10 mg mL⁻¹. The plates were incubated for 18-24 h at 37°C. The antibacterial activity was evaluated by measuring the zone of inhibition. The antibiotic streptomycin at 100 µg mL⁻¹ was used in the test system as positive control. The measured Zone of Inhibition (ZOI) was noted. The average of the zone of inhibition was obtained from three replicates of the isolated constituents as well as ethyl acetate extract.

Minimum Inhibitory Concentration Assay (MIC)

The Minimum Inhibitory Concentration (MIC) values were determined only with those bacteria that showed inhibitory zones greater than 20 mm (Demo *et al.*, 2005). *B. cereus* and *P. aeruginosa* have shown the maximum (>20 mm) zones hence have been selected for MIC assay. The dilutions 5, 3, 2, 1 and 0.1 mg mL⁻¹ were prepared in DMF. The assay was carried out and zone of inhibition was measured. The MIC values were determined as the lowest concentration of the constituent which completely inhibited the growth (Mazzanti *et al.*, 2000).

RESULTS AND DISCUSSION

Spectral data showed that the major constituents structurally resembled to that of alantolactone, isoalantolactone, and dihydroalantolactone. The plant *Inula racemosa* represent a broad spectrum of activity. All the isolated constituents and ethyl acetate extract inhibited the growth of bacteria at effective concentration of 200 µg well⁻¹ (10 mg mL⁻¹). Ethyl acetate extract was found to show inhibition against all the tested microorganisms except *Serratia marcescens* and *Shigella dysenteriae*. The isolated constituents of the plant showed moderate to high activity as antibacterial agent with two Gram positive and six Gram negative bacteria. The results of the antibacterial activity are reported in Table 1.

Table 1: Antibacterial activity of the isolated constituents from the root of *Inula racemosa*

Bacteria	Zone of inhibition (mm)				Standard Streptomycin
	Alantolactone	Isoalantolactone	Dihydroalantolactone	Ethyl acetate extract	
<i>Pseudomonas aeruginosa</i>	21	18	12	12	28
<i>Bacillus cereus</i>	24	17	13	09	27
<i>Serratia marcescens</i>	16	14	11	-	25
<i>Escherichia coli</i>	19	14	12	14	25
<i>Shigella dysenteriae</i>	16	12	10	-	24
<i>Salmonella typhi</i>	17	14	12	15	26
<i>Staphylococcus aureus</i>	18	13	13	10	22
<i>Klebsiella pneumoniae</i>	19	15	13	15	25

Maximum inhibition >20 mm, Moderate inhibition 16-20 mm, Poor inhibition 10-15 mm

The data analysis in Table 1 indicates that the tested constituents, ethyl acetate extract showed the significant results when compared with the standard streptomycin while alantolactone shown maximum inhibition of *Pseudomonas aeruginosa* and *Bacillus cereus* and moderate inhibition against the remaining tested bacteria. Of all the constituents of *Inula racemosa* the alantolactone had shown moderate inhibition of *Shigella dysenteriae*. In the earlier reports *Inula racemosa* has been used in China against bacillary dysenteriae (Tsewang, 1994). As our results suggest that the ethyl acetate extract of the plant has poor activity than the purified alantolactone constituents, which indicates the effectiveness of alantolactone in bacillary dysenteriae.

Isoalantolactone shows moderate inhibition against two bacteria *Pseudomonas aeruginosa* and *Bacillus cereus* and was found to show poor inhibition with the remaining bacteria. Dihydroalantolactone has shown poor inhibition against all the tested bacteria. The MIC values of alantolactone for *Bacillus cereus* and *Pseudomonas aeruginosa* was found to be $100 \mu\text{g mL}^{-1}$. These values shows the potential of the isolated constituents in low concentration which is comparable with the standard antibiotics. The data indicates that among the isolated constituents, alantolactone shows better activity profile as compare to the isoalantolactone, dihydroalantolactone and ethyl acetate extract.

The antibacterial activity profile of the isolated constituents when compared with the ethyl acetate extract shows that the activity depends on the pure form of the constituents. The results indicate that the degree of antibacterial activity is based on the varying chemical nature of the isolated constituents. The structural specificity and stereochemical aspects of the constituents might be playing a vital role in the better activity profile. The resistance of the bacteria to the current antibiotics necessitate the further studies on the isolated constituents to find out their safety and efficacy profile.

CONCLUSION

The present systematic investigation of the antibacterial activity of the constituents of *Inula Racemosa* have lead to the identification of more active antibacterial agents. The isolated sesquiterpene lactones represents a broad spectrum of activity against Gram positive and Gram negative bacteria. In the present study, the antibacterial activity profile of alantolactone against *Shigella dysenteriae* indicates its use in bacillary dysenteriae. The detail investigation of the alantolactone for the exact mode of action will be a major step in developing a novel therapeutic molecule for the antibacterial discovery.

REFERENCES

- Chopra, R.N., S.L. Nayar and I.C. Chopra, 1956. Glossary of Indian Medicinal plants, Council of Science and Industrial Research, Publications, New Delhi, India.
- Davis, J., 1994. Inactivation of antibiotics and the dissemination of resistance genes. *Sci.*, 264: 375-382.
- Demo, M., M.D. Oliva, M.L. Lopez, M.P. Zunino and J.A. Zygado, 2005. Antimicrobial activity of essential oils obtained from aromatic plants of Argentina. *Pharm. Biol.*, 43: 129-134.
- Dwivedi, S. and P.N. Somani, 1988. Cardioprotective effects of certain indigenous drugs in myocardial ischemia in rabbits. *Indian J. Exp. Biol.*, 26: 969-975.
- Mazzanti, G., N.T. Mascellino, L. Pattinelli, D. Coluccia, M. Manganario and L. Saso, 2000. Antimicrobial investigation of semipurified fractions of *Ginkgo biloba* leaves. *J. Ethnopharmacol.*, 71: 83-88.
- Moerman, D.E., 1986. Medicinal Plants of Native America. Ann Arbor., Univ. Michigan, Mus. Anthropol. Techn. Rep., 19: Vol. II .

- Mosquera, O.M., Y.M. Correa and J. Nino, 2004. Antibacterial activity of some Andean Colombian plants. *Pharm. Biol.*, 42: 499-503.
- Newman, D.J., G.M. Cragg and K.M. Snader, 2003. Natural products as sources of new drugs over the period 1981-2002. *J. Natl. Prod.*, 66: 1022.
- Okuda, T., 1986. *Encyclopedia of natural medicine*. Hirokqur, Tokyo, Japan.
- Rios, J.L., M.C. Recio and A. Villar, 1988. Screening methods for natural products with antimicrobial activity: A Rev. literature. *J. Ethnopharmacol.*, 23: 127-149.
- Satyavati, G.V., A.K. Gupta and N. Tandon, 1987. *Medicinal plants of India*. Indian Council of Medical Res., New Delhi, Vol. II.
- Singh, R.P., R. Singh, P. Ram and P.G. Batliwala, 1993. Use of pushkar-guggul, an indigenous antiischemic combination, in the management of ischemic heart disease. *Intl. J. Pharmacognosy*, 31: 147-160.
- Tan, R.X., H.Q. Tang, J. Hu and B. Shuai, 1998. Lignans and sesquiterpene lactones from *Artemisia sieversiana* and *Inula racemosa*. *Phytochemistry*, 49: 157-161.
- Tripathi, Y.B., P. Tripathi and B.N. Upadhyay, 1988. Assessment of the adrenergic beta-blocking activity of *Inula racemosa*. *J. Ethnopharmacol.*, 23: 3-10.
- Tripathi, Y.B. and P. Chaturvedi, 1995. Assessment of endocrine response of *Inula racemosa* in relation to glucose homeostasis in rats. *Ind. J. Exp Biol.*, 33: 686-689.
- Tsewang, J.T., 1994. *Tibetan Medicinal Plants*. 1st Edn., Tibetan Medical Publications, India.
- Wang, K., H. Liu, Y. Zhao, X. Chen, Z. Hu, Y. Song and X. Ma, 2000. Separation and determination of alantolactone and isovalantolactone in traditional Chinese herbs by capillary Electrophoresis. *Talanta*, 52: 1001-1005.