



RESEARCH ARTICLE

Phytochemical Screening and Antimicrobial Activity of *Ougeinia Oojeinensis* Leaves

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<p>Manuscript details</p> <p>Submitted on 10.04.2015 Revised on 15.05.2015 Accepted on 18.06.2015 © CJPS All right reserved</p> <p>Columbia Journal of Pharmaceutical Sciences: 2015; 2 (2); April-June, Pages-06-10</p>	<p>ABSTRACT:</p> <p>The present investigation was undertaken to evaluate the phytochemical and antimicrobial activity of the methanol and aqueous extracts of leaves of <i>Ougeinia oojeinensis</i>. The leaves extracts of <i>Ougeinia oojeinensis</i> were prepared using different solvents like petroleum ether, methanol and distilled water. The phytochemical screening of the leaves extracts was performed. The presence of alkaloids, glycosides, carbohydrates, steroids, polyphenol, saponins and terpenoids were indicated by the test conducted. The antimicrobial activity and minimum inhibitory concentration (MIC) of the methanol and aqueous extract of <i>Ougeinia oojeinensis</i> leaves was tested by agar diffusion method. Zones of Inhibition produced by both extract in a dose from 50 and 200 mg/ml against selected strains was measured and compared with those of standard drug ciprofloxacin. Both extract recorded significant activity against all the test bacteria. The lowest MIC was calculated for <i>C. albicans</i> with a concentration of 12.5 mg/ml with aqueous extract, while the highest MIC calculated was for <i>E. coli</i> (0.78 mg/ml) with methanol extract. The maximum antimicrobial activity was exhibited by methanol extract against all the microorganisms compared to be aqueous extract.</p>
<p>KEYWORDS:</p> <p><i>Ougeinia oojeinensis</i>, Phytochemical screening, Antimicrobial activity</p>	

1. INTRODUCTION:

Over the past 2 decades, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs and active compounds. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. The indigenous system of medicine namely Ayurvedic, Siddha and Unani has been in existence for several centuries¹. This system of medicine supports the need of more than 70% of population residing in the rural areas. Besides the demands made by

these systems as their raw materials, the demands of medicinal plants made by the modern pharmaceutical industries have also increased manifold². Since a long period of time, plants have been a valuable source of natural products for maintaining human health and infections control because, microbial infections pose a health problem throughout the world, and plants are a possible source of antimicrobial agents. Many of the herbs and spices used by humans to season food yield useful medicinal compounds. Microbial infections pose a health problem throughout the world, and plants are a possible source of antimicrobial agents. Medicinal plants contain active principles which can be used as an

alternative to cheap and effective herbal drugs against common bacterial infections³.

Ougeinia oojeinensis (Roxb.) Hochr (Fabaceae) known in Hindi as Tinsa and in Sanskrit as Ratha is a deciduous trees, found in the outer Himalayas and sub-Himalayan tracts from Jammu to Bhutan up to an altitude of 1500m and extending through the whole of northern and central India into the greater part of Deccan peninsula[4,5]. The extract of the whole plant *O. oojeinensis* were scientifically evaluated for anti-inflammatory and analgesic activities in previous studies. The 50% of ethanolic extract of stem bark has been reported exhibit antispasmodic action[6]. The hepatoprotective, in-vitro anti-inflammatory and wound healing activity of *O. oojeinensis* bark have been reported[7-9]. Phytochemical investigated on *O. oojeinensis* have reported the presence of lupeol, hydroxlupeol, betulin and isoflavanones such as dalbergioidin, homoferreirin and ougenin[10-12]. We report the results of screening for phytochemical and antimicrobial activity of methanol and aqueous extracts of *O. oojeinensis* leaves.

2. MATERIAL AND METHODS:

2.1 Plant materials

The leaves of *Ougeinia oojeinensis* were collected from Betul district, Madhya Pradesh, India, during the months of January and February 2007. The species was identified by the local people during the time of collection and later on authentication was made by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai, India. The leaves were shade dried, reduced to coarse powder and stored in airtight container till further use.

2.2 Preparation of extract

1 Kilogram of powdered drug was packed in Soxhlet apparatus and extracted with petroleum ether to defat the drug. Defatted powdered drug was then extracted with methanol. The methanol extract was separated and the marc was further extracted with distilled water. The solvents were removed by distillation and the last traces of solvent being removed under reduced pressure.

2.3 Preliminary Phytochemical studies

Preliminary phytochemical tests of various extracts of roots powder of *Hemidesmus indicus* were performed for phytochemical analysis of alkaloids, glycosides, carbohydrates, steroids, polyphenol, saponins and terpenoids^{13,14}.

2.3.1 Test for alkaloids

(a) Dragendorff's test: To 1 ml of the extract, add 1 ml of dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

- (b) Mayer's test: To 1 ml of the extract, add 1 ml of mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.
- (c) Hager's test: To 1 ml of the extract, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.
- (d) Wagner's test: To 1 ml of the extract, add 2 ml of wagner's reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

2.3.2 Test for saponins

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

2.3.3 Test for Glycosides

- (a) Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.
- (b) Baljet test: To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.
- (c) Keller-Killiani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.
- (d) Borntrager's test: Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammoniacal layer shows the presence of anthraquinone glycosides.

2.3.4 Test for carbohydrates and sugars

- (a) Molisch's test: To 2ml of the extract, add 1ml of α -naphthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

- (b) Fehling's test: To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars
- (c) Benedict's test: To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.3.5 Test for tannins and phenolic compounds

- (a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.
- (b) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.
- (c) The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

2.3.6 Test for flavonoids

- (a) The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.
- (b) Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, disappears on addition of an acid indicates the presence of flavonoids.
- (c) Shinoda's test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.
- (d) The extract is treated with sodium hydroxide, formation of yellow colour indicates the presence of flavones.
- (e) The extract is treated with concentrated H₂SO₄, formation of yellow or orange colour indicates flavones.

2.3.7 Test for steroids

- (a) Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of steroids.
- (b) Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H₂SO₄. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the

acid layer represents the steroidal components in the tested extract.

2.3.8 Test for triterpenoids

Noller's test: Dissolve two or three granules or tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink colour indicates the presence of triterpenoids.

2.4 Test microorganism

The antibacterial activity of extracts of plants was tested against four species of microorganisms: *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella sonnei*, and *Candida albicans*

2.5 Culture medium and inoculum preparation

High sensitivity testing agar (Hi-Media) was used for checking antimicrobial activity of methanol and aqueous extracts of leaves of *Ougeinia oojeinensis* against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella sonnei*, and *Candida albicans*. The microbial strains were cultured on the slants in the sterilized Laminar Air Flow from the pure culture. These cultured slants were incubated at 37 °C for bacterial growth for 2–3 days. High sensitivity testing agar was mixed at a concentration of 23.4 g/1000 ml in distilled water and autoclaved at 121 °C for 15 min. A loop full from pure culture of a bacterial strain was mixed in the 10 ml of Nutrient broth medium and incubated at 37 °C overnight and the activated culture was used for streaking onto the agar plates for antimicrobial sensitivity.

2.6 Agar well diffusion assay

The antibacterial activity of the ethanol and aqueous extracts of *Hemidesmus indicus* roots were determined by Agar well diffusion assay. 2.34 gm of high sensitivity testing agar was dissolved in 100 ml of distilled water and autoclaved at 121 °C for 15 min. Before transferring this medium in sterilized petri plates, it was allowed to cool and then was poured into the petri plates and allowed to solidify. After this, it was inoculated with activated culture using sterile cotton swabs. And the wells were created using sterile agar borer and the wells were filled by adding 25 µl of each extracts using micropipette and were incubated at 37 °C for 12–24 h. Three replicates were carried out for extract against each of the test organisms. Simultaneously, addition of the respective solvents instead of extracts was carried out as controls, while Ciprofloxacin (10 µg/ml) was used as a positive control. After incubation, the diameters of the zones of inhibition (ZOI) were measured in millimeters, and the mean values were tabulated¹⁵⁻¹⁸.

2.7 Minimum inhibitory concentration (MIC) Assay

The extracts that showed antimicrobial activity were subjected to minimum inhibitory concentration (MIC) assay by using serial two-fold dilution method. MIC was interpreted as the lowest concentration of the sample, which showed clear fluid without development of turbidity. 75 µl of sterile nutrient broth media was decanted into each well of a sterile 96 well micro plates. Highest concentration of extract was added at 75 µl to the first well. After mixing 75 µl was transfer to the second well the same procedure for the next well to attain dilution of 1/2, 1/4, 1/8, 1/32, 1/64, 1/128, 1/256, 1/512 and 1/1024. Inoculum solution at 1.5 µl was added to every well. Being incubated for 24 hr at 37 °C, the tubes were monitored for turbidity growth and non-turbidity as no growth. The minimum inhibitory concentration end point was the lowest concentration of drug extract at which there was no visible growth¹⁹.

3. RESULTS AND DISCUSSIONS:

Presence of classes of secondary metabolite may be a useful indicator of both efficacy and potential toxicity; hence test for the presence of phytochemical classes with known bioactivity was done. Results show that *O. oojeinensis* constitutes approximately 3.6%, 15.2% and 20.4% of petroleum ether extract, methanol and aqueous extract respectively. Methanol and aqueous extract contains all the classes of phytoconstituents while the petroleum extract lacked in bioactive constituents like Carbohydrates, glycosides, polyphenols and saponins (Table 1). On the basis of qualitative chemical test, it has been observed that chemically therapeutic compounds are present in sufficient amounts in the plants of *O. oojeinensis*. The methanol and aqueous extract contains maximum number of phytoconstituents along with flavonoids, phenolic content, saponin etc and it imparts antibacterial activity. Hence ethanol and aqueous extracts were selected for antibacterial activity.

Table 1: Phytochemicals present in extracts of *O. oojeinensis*

Test for	Petroleum Ether extract	Methanol extract	Aqueous extract
Alkaloids	-	+	-
Carbohydrates	-	+	+
Glycosides	-	+	+
Polyphenols	-	+	+
Saponins	-	+	+
Steroids	+	-	-
Triterpenoids	+	-	-

+ = present, - = absent

Table 2: Effect of methanol and aqueous extract of *O. oojeinensis* in disc diffusion antimicrobial bioassay

Zone of inhibition (mm)					
Microorganisms	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>Shigella sonnei</i>	<i>C. albicans</i>
Methanol extract of <i>O. oojeinensis</i>					
50 mg/disc	2.4	0.0	1.2	0.5	0.0
100 mg/disc	4.1	0.9	1.8	2.1	0.4
150 mg/disc	6.8	1.5	2.7	4.8	1.2
200 mg/disc	9.3	4.3	6.4	6.2	3.6
MIC (mg/ml)	0.78	6.25	3.12	3.12	6.25
Aqueous extract of <i>O. oojeinensis</i>					
50 mg/disc	0.8	0.0	0.0	0.0	0.0
100 mg/disc	1.7	0.0	0.9	0.0	0.0
150 mg/disc	3.4	0.6	1.8	1.2	0.0
200 mg/disc	5.2	1.8	3.2	2.8	1.1
MIC (mg/ml)	1.56	18.75	3.12	4.68	12.5
Ciprofloxacin					
5 µg/disc	2.2	2.5	1.3	0.0	---
25 µg/disc	3.8	4.1	2.8	1.1	---
50 µg/disc	6.1	7.4	4.1	1.9	---
100 µg/disc	8.4	10.3	6.3	4.5	---
MIC (µg/ml)	0.78	0.78	1.56	6.25	---
Ketoconazole					
5 µg/disc	---	---	---	---	1.7
25 µg/disc	---	---	---	---	2.4
50 µg/disc	---	---	---	---	5.6
100 µg/disc	---	---	---	---	8.2
MIC (µg/ml)	---	---	---	---	0.78

Minimum inhibitory concentration (MIC) and Zone of inhibition (ZOI) of both extracts of *O. oojeinensis* on gram positive and negative bacteria as well as a fungus, at different concentrations, by agar well diffusion method, was determined to access their antimicrobial effect. Methanol and aqueous extracts were active (weak to moderate) against all the microbes tested. The highest zone of growth inhibition was exhibited by methanol extract on *E. coli* giving a mean zone diameter of 9.3 mm when administered at a dose of 200 mg/ml. The lowest zone of growth inhibition was observed at same concentrations on *C. albicans* which gave a zone of inhibition measuring 1.1 mm with aqueous extract. The lowest MIC was calculated for *C. albicans* with a concentration of 12.5 mg/ml with aqueous extract, while the highest MIC calculated was for *E. coli* (0.78 mg/ml) with methanol extract. The antibacterial properties suggest that the phytoconstituents present in methanol extract are more potent than aqueous extract (Table 2), and also corroborate the use of *O. oojeinensis* in traditional medicine for itch and, common skin problems.

The result of antimicrobial studies shows that both methanol as well as aqueous extract exhibited significant activity, but, methanol extract was more active than aqueous extract.

4. REFERENCES:

- Bonjar G, Nik A, Heydari M, Ghasemzadeh M, Farrokhi P, Moein M, Mansouri S, Foroumadi A. Anti-Pseudomonas and anti-bacilli activity of some medicinal plants of Iran. DARU. 2003; 11: 157-163.
- Tepe B, Donmez E, Unlu M, Candan F, Daferera D, Vardar-Unlu G. Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret Aucher ex Benth.) and *Salvia multicaulis* (Vahl). J. Food Chem. 2004; 84: 519-525.
- Purohit P, Thakur Bais R, Sing P, Khan S. Assessment of antibacterial activity and phytochemical screening of *Hemidesmus indicus* root extracts. UK Journal of Pharmaceutical and Biosciences. 2014; 2(6): 67-72.
- Kirtikar KR, Basu BD. Indian medicinal plants. Vol. I. Dehradun, India, Oriental Longman Ltd, 1998: 756.
- Anonymous. The Wealth of India, Raw Material. Vol. VII. C.S.I.R. New Delhi, 1997; 193-197.
- Khare CP. Encyclopedia of Indian Medicinal Plants. Springer. 2004; 343.
- Sahu RK, Roy A. Hepatoprotective activity of ethanolic extract of bark of *Ougeinia oojeinensis* (Roxb.) Hochr in CCl₄ treated male rats. Pharmacologyonline. 2009; 2: 1-5.
- Sahu RK, Dewangan D, Roy A, Namdeo KP. Anti-inflammatory action of *Ougeinia oojeinensis* (Roxb.) Hochr. bark by HRBC membrane stabilization. Research Journal of Pharmacy and Technology. 2008; 1(01): 57-58.
- Sahu RK, Kulshrestha V, Kothiya S, Yadav P, Roy A. Healing potential of gel containing extract of *Ougeinia oojeinensis* on excision wounds in wistar rats. Journal of Global Pharma Technology. 2009; 2: 103-106.
- Mukherjee DK, Barua AK, Bose PK. Chemical Investigation of *Ougeinia dalbergioides* Benth. Science and Culture. 1963; 29: 151-152.
- Ghosh AC, Dutta NL. Chemical Investigation of *Ougeinia dalbergioides* Benth. Journal of Indian Chemical Society. 1965; 42(12): 831-835.
- Balakrishna S, Ramanathan JD, Seshadri TR, FRS, Venkataramani B. Special Chemical Components of the Heartwood of *Ougeinia dalbergioides* Benth. Proc. Royal Society London. 1962; 268A: 1
- Harbone JB. Phytochemical Methods: A guide to modern techniques of plant analysis. London: Chapman and Hall Ltd; 1984.
- Kenwat R, Prasad P, Sahu RK, Roy A, Saraf S. Preliminary phytochemical screening and in vitro antioxidant efficacy of fruit oil of *Martynia annua*. UK Journal of Pharmaceutical and Biosciences. 2014; 2(1): 16-22.
- Gupta AK, Ahirwar NK, Shinde N, Choudhary M, Rajput YS, Singh A. Phytochemical Screening and Antimicrobial Assessment of Leaves of *Adhatoda vasica*, *Azadirachta indica* and *Datura stramonium*. UK Journal of Pharmaceutical and Biosciences. 2013; 1(1): 42-47.
- Manohar P, Rajesham VV, Ramesh M, Kiran Kumar S, Prasanna JK. Pharmacognostical, Phytochemical and antimicrobial activity of *Bauhinia racemosa* leaves. Journal of Pharmaceutical Biology. 2011; 1: 10-14.
- Pelczar MJ, Chan ECS, Krieg NR. Microbiology, Int. Ed. Mcgraw Hill, New York. 1993; p. 578.
- Taylor RSL, Manandhar NP, Hudson JB, Towers GHN. Screening of selected medicinal plants of Nepal for antimicrobial activities. J Ethnopharmacol. 1995; 546: 153-159.
- Basri DF, Fan SH. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. Indian J Pharmacol. 2005; 37(1): 26-29.