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Phytochemical analysis and Antibacterial activity of *Dolichandrone falcata* (*Seem*), againstselected Human Pathogens.

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ABSTRACT

The present study aim is identification of maximum phytochemicals and its antibacterial effect against some selected multidrug resistant human pathogens. The selected plant *Dolichandrone falcata* Seem. Showedpresence of alkaloids, phenolic, Flavonoids, terpenoids, tannins, steroids but absence of mucilage, Tryosine and Tryptophan in all solvent extracts. The plant extracts proved to be very potent against selected human pathogens, which are resistant to multidrugs. The results obtained by antibacterial activity showed equivalent antibacterial zone of inhibition to standard antibiotic Amoxicillin.

Keywords: *Dolichandrone falcata*, Phytochemistry, Antibacterial activity,

INTRODUCTION

Phytochemicals are the constituents found in plants. The need of quality control is identification of biologically active compounds. Researchers also should focus for concentration determination of plant based drugs (Ganesan *et.al.*,2008). Aparna *et.al,* (2009) reported that plant possess chemical constituents having antioxidant activity through '*Dolichandroside*. The identified compounds or biomarkers are responsible for potential activity.

***Image .1:- Dolichandrone falcata* Seem.entireplant in Ganpat University Campus**

These are assuring for quality and identity of biomolecules. Herbal drugs are sources of bioactive compounds used mainly for medicinal purposes. (Dorobat, O.M. *et. al*.,2007., Madhu Babu Kasimala,*et. al.*, 2014.)

Dolichandrone falcata belonging to family Bignoniaceae is a medium size tree distributed in the central and southern parts of India.(Kirtikar KR and Basu BD. 1995). *Dolichondrone falcata*is a small or medium height or some time tall deciduous tree, having grayish colored bark. Leaflets are 5 to 6 inches .Leaves are compound. Oval, round or elliptical in shape. Flowers are white in colour. Flower stalk is 1/2 inch long. Sepal tube is 2-3 cm petals are free. Pods shows appearance like sheep horns so it is called as Medshingi. The flowering season is generally in month of May or June.

MATERIAL AND METHOD: *Dolichandrone falcata Seem.*pods collected from the Ganpat University Campus, Meshana, Gujarat. The accession number obtained by Prof and Head, Dept. of Botany, Dr. Dhabe, Dr. Babasaheb Ambedkar Marathwada University Aurangabad . The accession number is (0697). The collected pods washed and cleaned with $HgCl_2$, to make the pods microbes free. Pods get shade dry about twenty days. It gets stored in the form of powder in the amber colored bottle. The stored powder extracted by using different polar and non polar solvents. Ethanol, Methanol, petroleum ether, chloroform and Aqueous solvents.

About 250 gm. powder gets filled separately in thimble of soxhelt apparatus. It gets filled with respected solvents Ethanol, Methanol, Pet. Ether, Chloroform and Aqueous solvent. The assembly filled with 1000ml. of each solvent. The ratio of sample to solvent is 1:4 respectively. The obtained extracts stored in vial at 4°c temperature for further use.

2.1.Preliminary Phytochemical Test :- The Practical Pharmacognosy text book used as reference for preliminary phytochemical tests.(Khandelwal and Sethi 25th edition 2015)

2.1.1.Test For Carbohydrates :-

a. Molisch's test (General test):In the sample extract few drops of alpha-naphthol and 1ml of H_2SO_4 .Violet ring indicates presence of Carbohydrates.

b. Fehling's test:- When Fehling A and Fehling B solution mix at 1:1 ratio. Brick red colour indicate presence of Reducing sugar.

c. Benedicts test :-By addition of Bendicts Reagents and heating on water bath if red ppt indicates then it showed presence of reducing sugar.

d. Barfoed's test :- Mix equal volume of Barfoed's reagents with test solution. Red colour ppt does not observed which support absence of monosaccharides.

2.1.2. Test for sugars:-

a. Test for pentose sugar - add 2 drops of HCL in test solution , add crystals of phloglucinol. Red color absent indicate absence of pentose sugar.

b.Test for Hexose sugar :- Heat selwinoffs reagent and 1.5ml test solution in water bath for 1-2 min. Red colour absent .

2.1.3. Test For Mucilages :- Powdered drug material does not shows red colour with ruthenium red . It represents absence of mucilage.

2.1.4. Test For Proteins :-

a. Biuret Test:- mix 2 ml of test solution and add 4% NaOH and 1% cuso4 solution. If Violet colour appeare , it shows presence of proteins.

b. Millon's test :- Add 3 ml of millon's solution in test solution shows red colour, it shows presence of proteins.

2.1.5.Test For Alkalods:-

a. Dragendorff's test : Few drops of Dragendoffs reagent in test solution. If Orange or brown ppt is formed. It represents presence of alkaloids.

b. Mayers Test :- Few means 2-3 ml of test solution along with Mayers reagents. It gives ppt. It shows presence of alkaloids.

c. Hagers reagent :- Hagers reagents with test solution gives yellow ppt.

d. Wagners Test :- Few drops of Wagners Reagent in test solution,Gives yellow ppt.

2.1.6. Test For Flavonoids :-

a. Shinoda Test :- Extract along with ethanol and few drops of conc. HCL, then add 0.2g of magnesium ribbon crystals. Orange colour indicates presence of flavenoids.

b. Sulphuric Acid Test : Deep yellow colour represents presence of flavonoids.When added few drops of H_2SO_4 in test solution.

c. Lead Acetate Test :- Add small quantity of Lead Acetate solution in test sample gives yellow coloured precipitate.

d. Zinc Powder Test :-Adding Zn powder in conc. HCL . If Red colour observed indicates presence of Flavonoids.

2.1.7. Phenol Test :- By adding few drops along with test solution and following reagents :

a. 5% FeCl3 Solution :- Deep blue black colour colour.
b. Lead acetate solution :- White ppt.
c. Gelatin Solution :- white ppt.
d. Dil.Iodine Solution Test :- gives Red colour.
e. Dilute HNO3 Test :- It gives Reddish or yellow colour.

2.1.8. Test For Saponin : Foam Test :- By shaking of extract solution vigorously if persistent foam observed. It represents presence of saponin.

2.1.9. Test For Amino Acid; -Ninhydrin Test :- Add few drops of Ninhydrin solution and heat it . It gives purple or bluish colour indicate presence of amino acid.

2.1.10. Test for Tryosine :-Add 2 to 5 drops of Millons reagent in test solution, shows dark red color.

2.1.11.Test of Tryptophan :- Adding 0.5 ml glyoxalic acid and conc. H2SO4. Reddish violet ring appears at the junction of the two layers.

2.1.12. Test for Cardiac Glycosides: To 0.8 ml of the extract, 1ml of glacial acetic acid and two or three drops of ferric chloride were added. This was under layered with 0.5 ml of conc. sulphuric acid. Formation of brown ring at the interface represents the presence of cardiac glycosides.

2.1.13.Test for steroids: To 2 ml of plant extracst along with chloroform is added with few drops of concentrated sulphuric acid, brown ring indicates the presence of steroids.

2.1.14. Test for Tannins: To 1 ml of extract, added with 1 ml of 5% ferric chloride. Formation of dark blue or greenish black showed the presence of tannins.

2.1.15. Test for Coumarins: The few drops of 10% sodium hydroxide was added to 2ml of the extract. Formation of yellow colour indicates the presence of coumarins.

2.1.16. Anthraquinones: The few drops of 10% ammonia and 1ml of plant extracts solution was added, formation of pink color precipitate indicates the presence of anthraquinones.

2.1.17. Test for Phloba tannins: Few drops of dilute hydrochloric acid were added to 1ml of the plant extract.The appearance of reddish colour precipitate proved the presence of phloba tannins.

2.1.18.Tests for Glycosides :-

a.Liebermann's Test. :- The plant extracts added few drops of acetic acid and chloroform ,The mixture cooled and conc. H2SO4 added in few drops. Green color showed presence of glycosides.
b.Keller-Kiliani Test:- A 2ml plant extracts solution and glacial acetic acid 2ml with 1 drop of 2.0% $FeCl_3$. The conc. Sulphuric acid was added. A brown ring formed between the layers which showed the presence of cardiac glycosides.
c.Salkowski's Test. The extracts added with H_2SO4. A reddish brown color formed which indicated the presence of part of the glycoside.
2.1.19. Test for Terpenoids. The 1ml of plant extract and few drops of chloroform and evaporated on the water bath and add few drops of H_2SO_4. A grey color formed which showed the entity of terpenoids.

Collection of Bacterial Culture:

Gram positive and Gram negative human pathogens *Escherichia coli* (ATCC-25922), *Salmonella typhi* (MTCC-734), *Staphylococcus aureus* (ATCC- 25923) *Klebsiella pneumonia* (13883), *micrococcus luteus* (ATCC-9341) *Vibrio cholera* (17562), *pseudomonasaeruginosa* are used as a test organisms. The culture obtained from Clinical microbiology Department, Ghati Hospital Aurangabad. All the test organisms maintained on nutrient agar and subculture once in every two weeks. They are stored at 5º temperature.

RESULT AND DISCUSSION

3.1. Phytochemicals analysis:

The preliminary phytochemical screening of pod extracts of *D. falcata* reveals that alkaloids are present in methanol and ethanol and chloroform extracts. But it proved that methanol and

chloroform extract showed maximum concentration of alkaloids. The aqueous and Pet ether does not showed the presence of alkaloids.

Carbohydrates were absent in Ethanol and Pet. Ether extracts. But showed presence in other Methanol, Chloroform and Aqueous extracts. Pentose sugar present only in Methanol and Ethanol extracts but absent in other extracts. The tested Hexose sugar was found to be only in Ethanol and Chloroform extracts.

The mucilages were absent in all tested extracts. The proteins test found to be positive only in Ethanol and Chloroform extracts. Flavonoids present in all tested extracts except Pet. Ether. The phenolic compounds were also showed positive tests in all studied extracts except Pet. Ether. Saponin was obtained in aqueous, ethanol extracts. But it is absent in Methanol Pet. Ether and Chloroform extracts.

Terpenoids were present in all extracts. Tannins present in aqueous, methanol, ethanol and pet. Ether extracts. But absent in chloroform extract. The amino acids were present only in methanol and ethanol extracts, but absent in other three extracts of *D. falcata* .Cardiac glycosides were present in all tested extracts except aqueous. Tannins were present in all tested extracts except chloroform extract.

Glycosides were absent in Pet. Ether and Chloroform extracts but showed positive result in other three tested extracts. The terpenoids were present only in aqueous and methanol extracts.Tryosine and Tryptophan tests showed negative result in all tested extracts.

Steroids present in aqueous, methanol, chloroform extract and absent in ethanol extract. Cardiac glycosides obtained in methanol, ethanol, chloroform and Pet. Ether, but not in aqueous. Flavonoids are present in aqueous, methanol, ethanol, chloroform extracts but not in pet ether extract. Phenol is present in all extracts except aqueous.

Table 1: Phytochemical screening of various extracts of *Dolichandrone falcata Seem.*pods.

Chemical composition	Aqueous	Methanol	Ethanol	Chloroform	Pet. Ether
Test For Carbohydrates.	++	++	---	+	--
a.Molisch's test.					
b.Fehling's test	++	++	---	+	--
c.Benedicts test	++	++	---	+	--
d.Barfoed's test	++	++	---	+	--
Test for sugars:					
a. Test for pentose sugar	---	++	++	---	---
b.Test for hexose sugar	---	---	++	+	---

Test For Mucilages	---	---	---	---	---
Test For Proteins					
a. Biuret Test	---	---	++	+	---
b.Millon's test	---	---	++	+	---
Test For Alkalods					
a.Dragendorff's test	--	+++	+	+++	--
b. Mayers Test	--	++	++	+++	__
d.Wagners Test	--	+	+	++	__
Test For Flavonoids					
a. Shinoda Test	+	++	+	++	--
b.Sulphuric acid Test	++	+++	+	+++	--
c. Lead Acetate Test	+++	+	+	+++	--
d. Zinc Powder Test	+++	+	+	+++	--
Phenol Test					
a.5% FeCl3 Solution	+++	+	+	+++	--
b.Lead acetate solution	+++	+	+	+++	--
c.Gelatin Solution	+++	+	+	+++	--
d.dil.Iodine Solution Test	+++	+	+	+++	--
e.Dilute HNO3 Test :	+++	+	+	+++	--
Test For Saponin: Foam Test	+	__	+	__	--
Test For Amino Acid; Ninhydrin Test	--	++	++	--	---
Test of Tryptophan	--	--	--	--	--
Test for Cardiac Glycosides	--	+	+	+	+
Test for steroids	+	++	--	+	++
Test for Tannins	+	+	+	--	+
Test for Coumarins	+	+	--	+	--
Anthraquinones	--	---	---	---	--
Test for Phloba tannins	--	--	--	---	---
Tests for Glycosides					
Liebermann's Test.	++	++	++	--	--
Keller-Kiliani Test:-	++	++	++	---	---
Salkowski's Test.	++	++	++	--	--
Test for Terpenoids	++	++	++	++	++
Test for Tryosine	--	--	--	---	---

3.2 Antibacterial activity

The antibacterial assay of *D. falcata* against selected seven different bacterial pathogens proved the potent antibacterial activity. The methanol extract of *D. falcata* showed most effective activity against *E. coli*.(17mm). Aqueous extract showed less effective (10mm), Ethanol and Pet. Ether extracts were equal potential against *E. coli* but Chloroform did not show any activity against *E. coli.* The Methanol extract (18 mm) showed most effective against *P. aeruginosa.* The least effective extract was aqueous. Methanol extract followed by pet. Ether (17mm). Ethanol and Chloroform extracts of *D. falcata* showed moderate activity against *P. aeruginosa.* The same means Methanol extract (17mm) showed most active against *K. pneumoniae* bacterial pathogen.Pet. ether (10mm) Chloroform (14mm) and aqueous(12mm) extracts showed moderate activity against *K. pneumonia.* The *M. luteus* bacteria also more susceptible to methanol extract(16mm) of *D. falcata.* The Pet. etherextract(15mm) followed to methanol extract against *M. Luteus*.

The Ethanol and Aqueous extracts (11mm and 12mm respectively) showed moderate activity against *M. luteus* but not chloroform extract. The S. typhi pathogen is more susceptible to Pet. Ether (17mm) extract. It was followed by chloroform (16 mm) extract.The methanol and ethanol (15mm) showed same inhibition zone against *S. typhi* bacteria. The Methanol and Ethanol extract (15mm) was again effective against *V.cholerae* .The Pet. Ether and Chloroformextracts(14mm) weresimilar zone of inhibition against *V.cholera.* The aqueous extract of *D. falcata* was ineffective against *V.cholera.* In *S. aureus* pathogen, here Methanol extract was did not show any activity. The aqueous extract proved to be more potent against *S. aureus*. The Pet. Ether, Chloroform and Ethanol extracts showed moderate activity against *S. aureus* bacterial pathogens. The negative control DMSO was inactive against all tested bacteria. The Amoxicillinis broadspectrum antibiotics used as positive control and was effective against selected pathogens.The result obtained are shown in below image.

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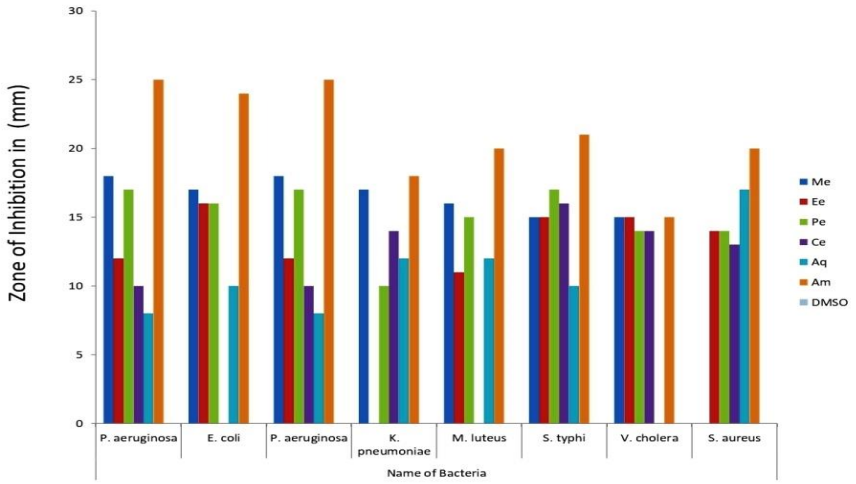
Image. 2. The Antibacterial Activity of *Dolichandrone falcata* pods.

Sr. No.	Name of bacteria	Zone of Inhibition in (mm)						
		Me	**Ee**	**Pe**	**Ce**	**Aq**	**Amoxicillin**	**DMSO**
1	*E. coli*	**17**	16	16	nil	10	24	nil
2	*P. aeruginosa*	**18**	12	17	10	08	25	nil
3	*K. pneumoniae*	**17**	Nil	10	14	12	18	nil
4	*M. luteus*	**16**	11	15	nil	12	20	nil
5	*S. typhi*	15	15	**17**	16	10	21	nil
6	*V. cholera*	**15**	**15**	14	14	nil	15	nil
7	*S. aureus*	nil	14	14	13	**17**	20	nil

Me- Methanol ,Ee- Ethanol, Pe- Petrolium Ether, Ce-Chloroform, Aq- Aqueous.



Gram positive and Gram negative human pathogens *Escherichia coli* (ATCC-25922) , *Salmonella typhi (*MTCC-734) ,*Staphylococcus aureus* (ATCC- 25923) *Klebsiella pneumonia* (13883), *micrococcus luteus* (ATCC-9341) *Vibrio cholera* (17562), *pseudomonas aeruginosa* (isolated from burn patients injury) tested in different solvent extracts of *Dolichandrone falcata Seem.*pods. Among all tests Methanol extract showed excellent inhibition zone.

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