

## Chemical Investigation and Antimicrobial Activity of *Rhoeo Discolor* Hance (Mee-Gwin-Gamon) Leaves

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### Abstract

The leaves of *Rhoeo discolor* Hance (Mee-gwin-gamon) have been chosen to investigate antimicrobial activity. Preliminary phytochemical examination of sample showed the presence of  $\alpha$ -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, starch and steroids by standard method. Alkaloids, cyanogenic glycosides, organic acids, reducing sugars, saponins, tannins and terpenoids were absent. The extractable matter contents were obtained by extraction with different polarity of solvents such as water (13.17 %), ethanol (12.08 %), ethyl acetate (1.44 %) and petroleum ether (1.17 %). The antimicrobial activity of petroleum ether, ethyl acetate and watery extracts of *Rhoeo discolor* Hance (Mee-gwin-gamon) leaves were determined by agar well diffusion method. These extracts were tested against six different microorganisms such as *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Staphylococcus aureus*. Among the tested extracts, only watery extract showed the antimicrobial activity against all tested microorganisms (27 mm- 36 mm). Although all the extracts inhibited (22 mm- 33 mm) against *Escherichia coli*, standard medicine (chloramphenicol) did not show.

**Keywords:** *Rhoeo discolor* Hance (Mee-gwin-gamon), phytochemical constituents, extractable matter, six microorganisms and antimicrobial activity

### Introduction

Commelinaceae is a family of herbaceous flowering plants with many species used in ethnobotany, particularly in South America (Tan, 2014). *Rhoeo discolor* are generally grown in soil that is moist, which came from India and West Mexico. Medicinal plants are the oldest known health care products used for the treatment of various ailments. The medicinal plants are gaining popularity as alternative and complementary therapies around the world because human body is more accustomed to natural products. *Rhoeo discolor* showed a high content of these metabolites such as flavonoids, carotenoids, anthocyanins, ferulic acid, chlorogenic acid, vanillic acid and also p-coumaric acid and steroidal compounds (Rosales, 2008). Lots of plants *Rhoeo discolor* that provide vitamins and fiber is good for the body such as an excellent plant for the source in medicine to cure a disease.

### Materials and Methods

#### Collection of leaves sample

The leaves of *Rhoeo discolor* Hance (Mee-gwin-gamon) were collected from Kyauk Taw Township, Rakhine State. The scientific name of the plant was identified by the authorized botanist at the Department of Botany, Sittway University.

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Figure 1. Nature of *Rhoeo discolor*

### **Phytochemical Test for Classification of Compounds in Plant Material by Standard Method**

The phytochemical examination of plant is one of the important experiments because it provides classification of groups of chemical substances present in it. The preliminary phytochemical examination by standard method indicates the chemical constituents of the leaves of *Rhoeo discolor* (Mee-gwin-gamon) (Trease and Evans; 1980; Robinson, 1983; Marini-Bettolo *et al.*, 1981).

### **Determination of Extractable Matter Contents in the Leaves of *Rhoeo discolor* Sample**

Each dried powdered sample (5 g) was extracted with 20 mL of PE (60-80 °C) for 6 hrs by extracted by using percolation method at room temperature and filtered. The filtrate was concentrated by removal of the solvent by distillation to give the respective pet-ether crude extract. The same procedure was used to extract ethyl acetate, 95 % ethanol and water.

### **Screening of Antimicrobials Activity of the Leaves of *Rhoeo discolor* Sample**

Antimicrobial screening of crude extracts of leaves of *Rhoeo discolor* were carried out by agar well diffusion method. The extracts were tested on six species of microorganisms such as *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescense* and *Staphylococcus aureus*. The discs containing crude extract of the leaves of *Rhoeo discolor*. Hance was placed in clockwise position on the prepared agar plate. The petri dish was then incubated at 37 °C for 48 hrs, and the diameters of clear inhibition zones around the hole, were measured. The antimicrobial activity was compared with the standard antibiotic disc (chloramphenicol) as control (Hnin Hnin Aye, 2002).

## **Results and Discussion**

### **Phytochemical Investigation of Plant Sample by Standard Method**

The leaves of *Rhoeo discolor* (Mee-gwin-gamon) were collected from Kyauk Taw, Township, Rakhine State. The leaves were washed with water and air dried at room temperature. The dried leaves were stored in the airtight containers to prevent moisture changes and contamination.

Preliminary phytochemical investigation of sample showed the presence of  $\alpha$ -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, starch and steroids by standard method. Alkaloids, cyanogenic glycosides, organic acids,

reducing sugars, saponins, tannins and terpenoids were absent that are shown in table 1.

**Table 1. Results of Phytochemical Investigation of Leaves of *Rhoeo discolor***

No.	Type of compounds	Extracts	Test reagents	Observation	Remark
1.	Alkaloids	1 % HCl	Mayer reagent Dragendorff's reagent Wagner's reagent Sodium picrate	No white ppt. No orange ppt. No brown ppt. No yellow ppt.	- - - -
2.	$\alpha$ -amino acids	H <sub>2</sub> O	Ninhydrin	Purple spot	+
3.	Carbohydrates	H <sub>2</sub> O	10 % $\alpha$ -naphthol & conc. H <sub>2</sub> SO <sub>4</sub>	Red ring	+
4.	Cyanogenic glycosides	H <sub>2</sub> O	Conc. H <sub>2</sub> SO <sub>4</sub> and sodium picrate	No brick red color	-
5.	Flavonoids	EtOH	Conc. HCl and Mg turning	Pink color	+
6.	Glycosides	H <sub>2</sub> O	10 % lead acetate	White ppt.	+
7.	Organic acids	H <sub>2</sub> O	Bromocresol	No ppt.	-
8.	Phenolic compounds	H <sub>2</sub> O	5 % FeCl <sub>3</sub>	Deep blue color	+
9.	Reducing sugars	Dil. H <sub>2</sub> SO <sub>4</sub>	Benedict's solution	No yellowish brown color	-
10.	Saponins	H <sub>2</sub> O	Distilled water	NO frothing	-
11.	Starch	H <sub>2</sub> O	Iodine solution	Greenish blue ppt.	+
12.	Steroids	PE	Acetic anhydride & conc. H <sub>2</sub> SO <sub>4</sub>	Blue color	+
13.	Tannins	H <sub>2</sub> O	2 % NaCl, gelatin	No ppt.	-
14.	Terpenoids	CHCl <sub>3</sub>	Acetic anhydride & conc. H <sub>2</sub> SO <sub>4</sub>	No red color	-

(+) Presence

(-) Absence

ppt. precipitate

**Extractable Matter Contents of the Leaves of *Rhoeo discolor***

The extractable matter contents of the leaves of *Rhoeo discolor* were obtained by extraction with different polarity of solvents such as water, ethanol, ethyl

acetate and petroleum ether and in order of 13.17 % > 12.08 % > 1.44 % > 1.17 % respectively that are tabulated in table 2.

**Table 2. Results of Extractable Matter Contents of leaves of *Rhoeo discolor* Hance**

No.	Solvent used	Extractable matter (%)
1.	Petroleum ether	1.17
2.	Ethyl acetate	1.44
3.	Ethanol	12.08
4.	Water	13.17

**Screening of Antimicrobial Activity of Different Crude Extracts by Agar Well Diffusion Method**

The various crude extracts of leaves of *Rhoeo discolor* Hance (petroleum ether, ethyl acetate, 70 % ethanol and watery) were tested on six species of microorganisms such as *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescense*, *Staphylococcus aureus* by using agar well diffusion method. Agar well diameter is 6 mm. The larger the diameter, the greater is the antimicrobial activity. From the experiment results, petroleum ether and ethyl acetate extracts did not show antimicrobial activity but ethanol and watery extracts showed antimicrobial activity against *Agrobacterium tumefaciens*, *Bacillus subtilis* and *Staphylococcus aureus* (14 mm- 28 mm). It was found that watery extract (28.8 mm) has more potent antimicrobial activity than the other extracts. Although the watery extract inhibited (14.3 mm) against *Escherichia coli*, the standard medicine (chloramphenicol) did not show that are presented in figure 2 and table 3.

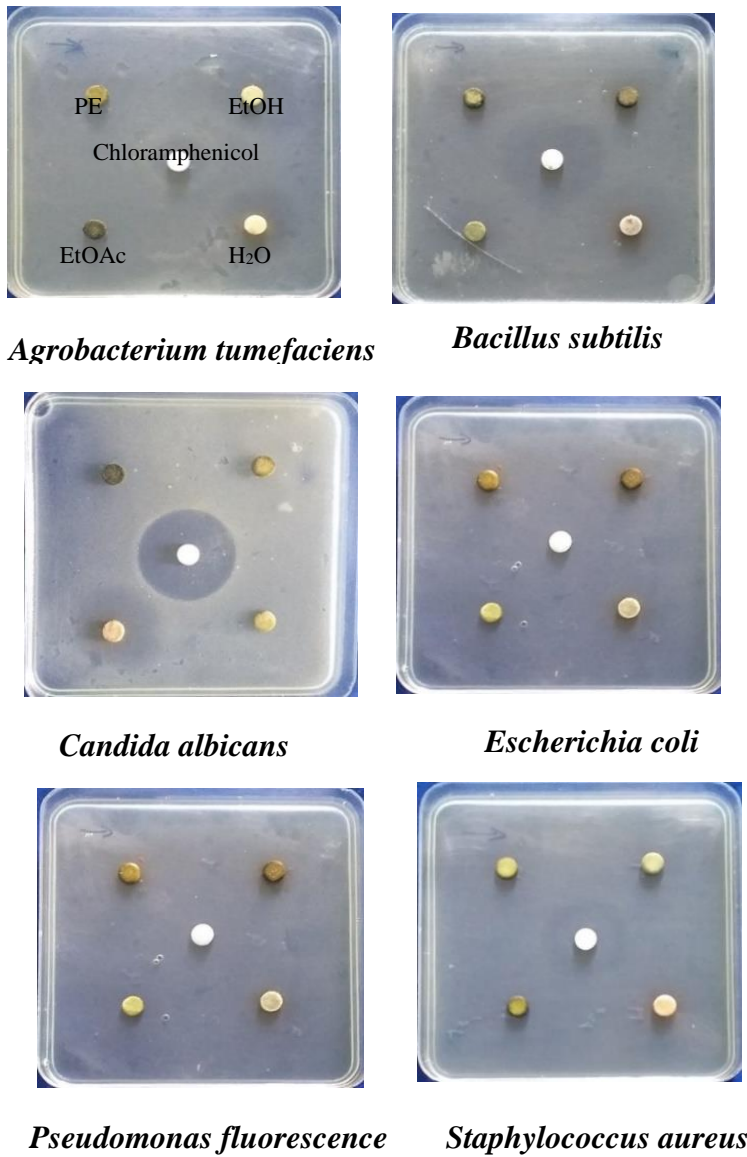


Figure 2. Inhibition zones of different crude extracts from the Leaves of *Rhoeo discolor* Hance by agar well diffusion method

**Table 3. Antimicrobial Activity of Crude Extracts of Leaves of *Rhoeo discolor* Hance**

No	Tested microorganisms	Inhibition Zone Diameter (mm)				
		PE	EtOAc	EtOH	H <sub>2</sub> O	Chloramphenicol
1.	<i>Agrobacterium tumefaciens</i>	-	38.2	-	27.6	24.0
2.	<i>Bacillus subtilis</i>	29.5	21.31	23.7	30.0	40.2
3.	<i>Candida albicans</i>	-	-	-	31.5	35.2
4.	<i>Escherichia coli</i>	22.2	33.4	29.3	33.5	-
5.	<i>Pseudomonas fluorescense</i>	-	-	28.0	36.2	39.0
6.	<i>Staphylococcus aureus</i>	-	49.7	-	36.3	33.5

Agar well diameter - 6 mm, (-) No activity

EtOH = Ethanol

EtOAc = Ethyl acetate

PE = Petroleum ether

H<sub>2</sub>O = Watery

### Conclusion

From the overall assessment of the phytochemical investigations and antimicrobial activity of leaves of *Rhoeo discolor* Hance (Mee-gwin-gamon), the following inferences could be deduced. The phytochemical investigations of the sample showed the presence of  $\alpha$ -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, starch and steroids by standard method. Alkaloids, cyanogenic glycosides, organic acids, reducing sugars, saponins, tannins and terpenoids were absent in the sample. The extractable matter contents were obtained by extraction with different polarity of solvents such as water (13.17 %), ethanol (12.08 %), ethyl acetate (1.44 %) and petroleum ether (1.17 %). The antimicrobial activity of the polar and non-polar extracts was screened by using agar well diffusion method on six microorganisms. Among the extracts, watery extract has antimicrobial activity against all tested microorganisms (27 mm- 36 mm). Although all the extracts inhibited (22 mm- 33 mm) against *Escherichia coli*, standard medicine (chloramphenicol) did not show. In present study, *Rhoeo discolor* extracts may be used as an antimicrobial agent and a source of medicine for treatment of diseases related to microbial infection.

### Acknowledgements

We are greatly indebted to Rector and Pro-Rectors from Dagon University for their great kindness to do this conference. We also wish to mention our sincere thanks to Dr Tin Moe Aye (Professor and Head) and Dr Yee Yee Thu (Professor), Department of Botany, Dagon University, for their strong efforts to complete 4<sup>th</sup> Myanmar - Korea Conference on Plants Tissue Culture and Genetics hosted by Jeonbuk National University Korea.

### References

- Hnin Hnin Aye. (2002). *Antituberculosis Activities and Chemical Investigation of Myanmar Traditional Medicine Used for the Treatment of Tuberculosis*. PhD Dissertation, Department of Chemistry, University Yangon
- Marini-Bettolo, G. B., M. Nicoletti and M. Patamia. (1981). "Plant Screening by Chemical and Chromatographic Procedure Under Field Conditions". *J. Chromato.*, **213(1)**, 113-128
- Robinson, T. (1983). *The Organic Constituents of Higher Plants*, North Amherst, 5<sup>th</sup> Ed., MA : Cordus Press, 285-286
- Rosales, R. T., M. D. Garza, C. C. A, M. M. Rodriguez, S. F. Fattel, E. Popoca and S. T. Villa. (2008). "Aqueous Crude Extract of Rhoecus Discolor, A Mexican Medicinal Plants, Decreases the Formation of Liver Preneoplastic Foci in Rats". *Journal of Ethnopharmacology*, **115(3)**, 381-386
- Tan, J., W. Yap, S. Tan, Y. Lim and S. Lee. (2014). "Antioxidant Content, Antioxidant Activity of Five Plants from the Commelinaceae Family". *Antioxidants*, **3(4)**, 758-769
- Trease, G. E. and W. C. Evans. (1980). *Phytochemistry, Introduction and General Methods. Pharmacognosy*, London, Bailliere Tindall, **11**, 45-50