Phytochemical Analysis andAntimicrobial Screening in Leaf Extracts of *Mangifera indica* cv. Sein Talone

Khin Myo San[1], Ohmmar Than[2]

Abstract

This research is carried out to evaluate the phytochemical analysis and antimicrobial screening of the leaves of *Mangiferaindica* cv. Sein Talone.The phytochemical analysis was done by using the procedure of Harbone method (1984). Antimicrobial screening was carried out by the using of agar well diffusion method (Balouiri *et al.* 2015). The result of phytochemical analysis showed the presence of glycosides, phenolic compounds, tanins, saponins, reducing sugars, flavonoids and alkaloids.The antimicrobial screening revealed theinhibiting zone that ethyl acetate extract on*Agrobacterium tumefaciens* is 15.59 mm; acetone extract on *A. tumefaciens* is 17.14 mm, *Bacillus pumilus* is 16.90 mm; methanol extract on *A. tumefaciens* is 18.48 mm; ethanol extract on *A. tumerfaciens* 22.06 mm, *B. pumilis* is 18.83 mm, *B. subtilis* is 27.09 mm, *Candidaalbicans* is 19.85 mm and *Saccharomyces cerevisiae*is 19.67 mm; water extract on *A. tumefaciens* is 13.39 mm. Therefore, this analysis concluded that the leaves of*Mangifera indica* cv. Sein Talone could be used in curing of fever, candidiasis, burn and wound diseases. It also used to prevent plant diseases and food spoilage.

Keywords: Phytochemical, Antimicrobial,Leaf Extracts

Introduction

Mangifera indica cv. Sein Talone belongs to the family Anacardiaceae. The leaves are simple, oblong-lanceolate with entire margins, midrib prominent,unicostate and reticulate venation, inflated basally petioles. Mango leaves are full of healing and medicinal properties. The mango leaves are known to use for the treatment of diabetes, hypertension, kidney disease, dysentery, restlessness, respiratory problems, earache, burns, hiccups and as stomach tonic etc. (Parvez 2016). Phytochemical analysis of the leaves of *Mangifera indica* cv. Sein Talone was carried out to identify the bioactive constituents. The standard methods have been used to analyse the presence of glycosides, phenolic compounds, tannins, saponins, reducing sugars, flavonoids and alkaloids.Antimicrobial activities of aqueous, petroleum ether, ethyl acetate, acetone, methanol and ethanol extracts of *Mangifera indica* cv. Sein Talone leaves were carried out by using of eight test organisms such as *Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus*, *Candidaalbicans* and *Saccharomyces cerevisiae*.

Ethanopharmacological data are one of the common useful criteria in drug discovery. Therefore, the study of phytochemical and antimicrobial activity of *Mangifera indica* cv. Sein Talone is carried out to detect the phytochemical compositions in leaves, to inform the treatments of leaves on diseasesandto know the effects of leaves on pathogenic microorganisms.

Materials and Methods

Collection of plant sample

Fresh samples of *Mangifera indica* cv. Sein Talonewas collected from Department of Botany, Kyaukse University Campus, during the period between June to August, 2019. The collected specimens were photographed to record the data and identified with the help of literatures by using of Backer (1963), Dassanayake

[1] Associate Professor, Department of Botany, Kyaukse University
[2] Lecturer, Department of Botany, Kyaukse University

(1996).The leaves were washed with tap water in order to remove the dust. Collected plant materials were then dried under the shade and ground to get powdered by using grinding mill andstored in air tight containers to prevent moisture changes and contamination.

Figure1.Powdered of leaves*Mangifera indica*cv. Sein Talone

Preparation of extract

About 5g powdered material of plant species was soaked in 50 mL solvent of Petroleum ether, Ethyl acetate, Acetone,Methanol, Ethanoland Water for five days, shaked well twice a day and then filtered. The filtrates were then evaporated under reduce pressure to obtain a gummy residue (Ankit,*et al.*2012). All extracts were stored in sterile glass bottle at room temperature until screened.

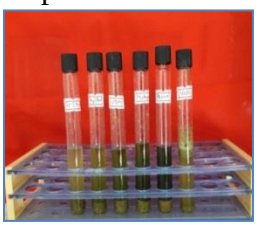

Figure 2. Mixture of leaves powdered andvarious solvents

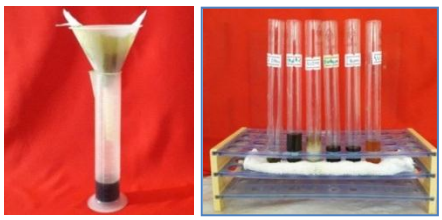

Figure 3. Filtered of various solvents of*Mangiferaindica*

Preliminary phytochemical analysis of *Mangifera indica* cv. Sein TaloneLeaves

For preliminary phytochemical analysis, the air-dried powders of the leaves were used. Test for glycosides, phenolic compounds, tannins, saponins, reducing sugars, flavonoids and alkaloids were analysed by using various solvents. These results were carried out according to themethod of Herbone (1984).

Test for Glycosides: The sample powder (2 g) was boiled with distilled water for about 10 minutes, allowed to cool, and filtered. The filtrate was treated with 10 % lead acetate solution. The appearance of white or yellow precipitate was obtained, which indicates the presence of glycoside.

Test for Phenolic Compound: The sample (2 g) was boiled with water for about 10 minutes allowed to cool, and filtered. The filtrate was treated with 10 % ferric chloride solution. A purplish colour indicates the presence of phenolic compound.

Test for Tannin: The sample powder (2 g) was boiled with distilled water for about 10 minutes, allowed to cool, and filtered. The filtrate was added few drops of 1 % $FeCl_3$. The brownish green was observed, indicating tannin may be present.

Test for Saponin: The sample (2 g) was boiled with distilled water for about 10 minutes allowed to cool, and filter. The filtrate was shaken. Forth was observed, indicating saponins may be present.

Test for Reducing Sugar: The sample powder (2 g) was boiled with distilled water for about 10 minutes, allowed to cool, and filtered. The aqueous filtrate was boiled with Benedict's solution in a test tube. Reddish brown color solution was obtained, which indicates the presence of reducing sugar.The aqueous filtrate was boiled with

Fehling solution in a test tube. If the brick red precipitate was obtained, it indicates the presence of reducing sugar.

Test for Flavonoids: Diluted hydrochloric acid (5 to 10 drops) was added to 0.5 ml of ethanolic extract of sample and then three pieces of magnesium were added to the solution. It was boiled for a few minutes and reddish brown colour shows the presence of flavonoid.

Test for Alkaloids: Small amount of crude sample was heated with 1% HCl and 5 drops of Wagner's reagent was added. The reddish brown precipitate was obtained and it indicates the presence of alkaloids.Small amount of crude sample was heated with 1% HCl and 5 drops of Dragendroff's reagent was added. The orange precipitate was obtained and it indicates the presence of alkaloids.

Antimicrobial screening

The preliminary study for antimicrobial screening was carried out by agar well diffusion method (Balouiri,*et al.*2015).

The different solvent were subjected to antimicrobial screeningagainst on *Agrobacterium tumefaciens, Bacillus pumilus, B. subtilis, Candida albicans,Escherichia coli, Pseudomonas fluorescens, Saccharomyces cerevisiae and Staphylococcus aureus.*Assay medium was prepared in conical flask in accordance to the directions provided by the manufacturer. The media alongwith petri dishes, pipette and metallic borer were sterilized in autoclave for 15 minutes at 121°C and 15 psi pressure. The media was poured intoPetri dishes under aseptic condition. All of the six bacterial strains and two yeasts were obtained from Biotechnological Resource Development Center(BDC), Pathein University.Microbial culture was inoculated on assay medium. Each of eight kinds of test organisms were spread on the solidified agar media, then 8 mm wells were punched in the agar media by using sterile metallic borer.Stock solutions of crude extract at concentration of 20µLof *M. indica* leaf extracts were added into respective wells.The petri dishes were incubated at 37°C for 24 hours. After 24 hours antibacterial screening were measured as diameterof the zones of inhibition.

Preparation of Assay medium (used for test organisms)

Glucose	-	1.0 g	Agar	-	1.5 g
Yeast extract	-	0.3g	Peptone	-	0.2g
KH_2PO_4	-	0.001g	$MgSO_4.7H_2O$	-	0.001 g
KNO_3	-	0.001g	Distilled water	-	100 mL
pH	-	7			

Table 1 Test organisms used in antimicrobial activities (NITE)

No	Test Organisms	Infections
1	*Agrobacterium tumefaciens* (IFO 5431)	Plant diseases
2	*Bacillus pumilus* (IFO 905771)	Wound and burn infection
3	*Bacillus subtilis* (IFO 90571)	Fever
4	*Candida albicans* (NITE 09542)	Candidiasis
5	*Escherichia coli* (AHU 5436)	Diarrhoea
6	*Pseudomonas fluorescens* (IFO 94307)	Rice diseases
7	*Saccharomyces cerevisiae* (NITE 52847)	Food spoilage
8	*Staphylococcus aureus* (AHU 8465)	Boils and food poisoning

Results

Preliminary phytochemical investigation of the powdered leaves of *Mangifera indica*

The results of these tests confirmed the presence of leaves of *Mangifera indica* cv. Sein Talone showed that the presence of glycosides, phenolic compounds, tannins, saponins, reducing sugars, flavonoids and alkaloids,. The results were shown in Figure 5 and Table 2.

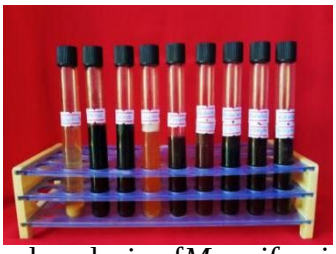

Figure (5)Phytochemical analysis of*Mangiferaindica*cv. SeinTalone

Table 2 Preliminary phytochemical investigation of powdered leaves of *Mangifera indica* cv. Sein Talone

No	Tests	Extracts	Test Reagents	Observations	Results
					Leaves
1	Glycosides	DW	10% lead acetate solution	White ppt.	+
2	Phenolic compounds	DW	3%$FeCl_3$ solution	Purple	+
3	Tannins	DW	1 drop of 1%$FeCl_3$ solution	BrownishGreen	+
4	Saponins	DW	Distilled water	Frothing	+
5	Reducing sugars	DW	Benedicts solution	Reddish brown	+
6			Fehling solution	Brick red ppt	-
7	Flavonoids	Ethanol	dil HCL 3 pieces of Mg	Reddish brown ppt	+
8	Alkaloids	Ethanol	1% HCl, 5 drops of Wagner's reagent	Reddish brown	+
9			Dragendroff's reagent	Orange	+

(+) Present(-) Absent

Antimicrobial Screening in Leaf Extracts of *Mangifera indica*

The investigation of various extracts of *Mangifera indica* cv. Sein Talone was against on antimicrobial activities. The results were shown in table 3 and figure 6 to 13.

Table 3 Antimicrobial activities of various solvents (Control) (Diameter zones of inhibition in mm)

No	Test organisms	Petroleum ether	Ethyl acetate	Acetone	Methanol	Ethanol	Water
1	*A. tumefaciens*	-	+	-	-	+	-
2	*B. pumilus*	-	-	-	-	-	-
3	*B. subtilis*	-	-	-	-	+	-
4	*C. albicans*	-	-	-	-	-	-
5	*E. coli*	-	-	-	-	+	-
6	*P. fluorescens*	-	-	-	-	-	-
7	*S. cerevisiae*	-	-	-	-	-	-
8	*S. aureus*	-	-	-	-	-	-

Agar well diameter = 8mm

Table 4 Antimicrobial Activities of Various Solvents (Diameter zones of inhibition in mm)

No	Test organisms	Petroleum ether	Ethyl acetate	Acetone	Methanol	Ethanol	Water
1	*A. tumefaciens*	-	15.59 mm	17.14 mm	18.48 mm	22.06 mm	13.39 mm
2	*B. pumilus*	-	-	16.90 mm	-	18.83 mm	-
3	*B. subtilis*	-	-	-	-	27.09 mm	-
4	*C. albicans*	-	-	-	-	19.85 mm	-
5	*E. coli*	-	-	-	-	-	-
6	*P. fluorescens*	-	-	-	-	-	-
7	*S. cerevisiae*	-	-	-	-	19.67 mm	-
8	*S. aureus*	-	-	-	-	-	-

Agar well diameter = 8mm

A.tumefaciens=Agrobacterim tumefaciens *B. pumilus= Bacillus pumilus*
B. subtilis = Bacillus subtilis *C .albicans= Candida albicans*
E. coli = Escherichia coli *P.fluorescens=Pseudomonas fluorescens*
S. cerevisiae= Saccharomyces cerevisiae *S. aureus=Staphylococcus aureus*

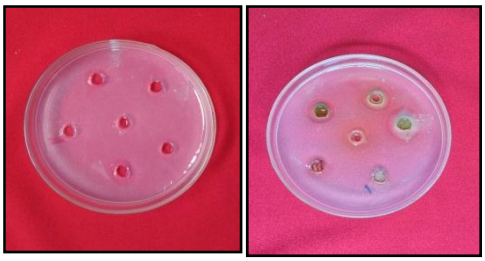

Figure (6)*Agrobacterium tumefaciens*

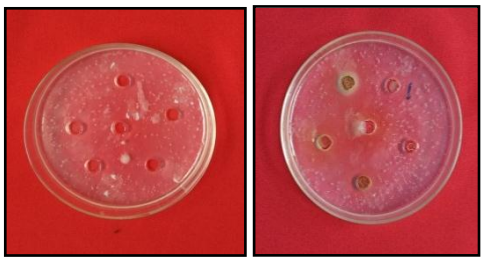

Figure (7)*Bacillus pumilus*

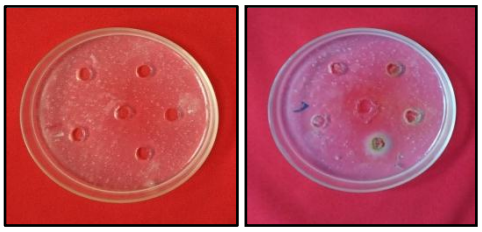

Figure (8)*Bacillus subtilis*

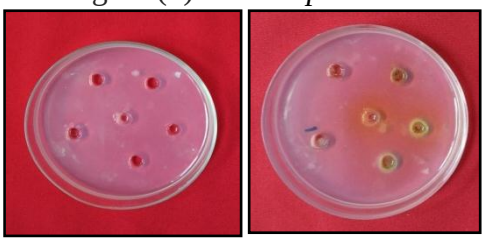

Figure (9)*Candida albicans*

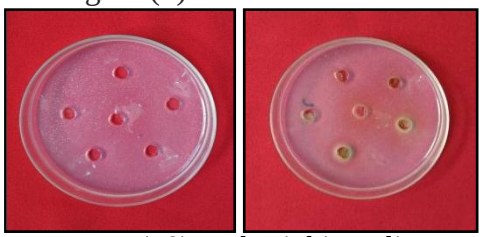

Figure (10)*Escherichia coli*

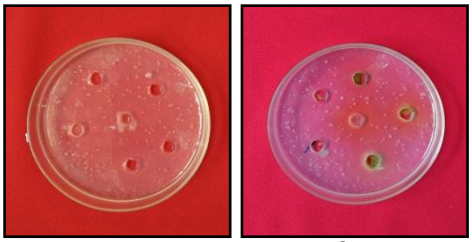

Figure (11)*Pseudomonas fluorescens*

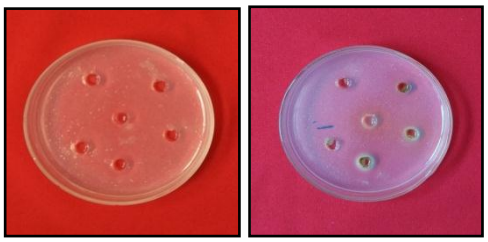

Figure (12)*Saccharomyces cerevisiae*

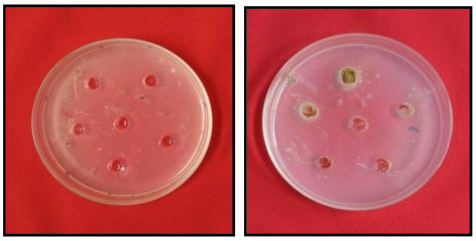

Figure (13)*Staphylococcus aureus*

Discussion and Conclusion

The phytochemical analysis carried on *Mangifera indica*leaf extract showed the presence of glycosides, phenolic compounds, tannins, saponins, reducing sugar, flavonoids and alkaloids. Alkaloids can be used in the treatment of malaria, cold, cough, hypertension, diabetes and cancer. Phenols are used as an antiseptic, analgesic and cosmetics. Tannins are used as astringent, dysentery, and diarrhea. Saponins are used for the treatment of arteriosclerosis and hypertension. Glycosides have antibacterial, antifungal, anti-inflammatory, antioxidant, antiviral and anticancer activities. Reducing sugar provides energy source for human body. Flavonoids are reduced risk of cancer, heart disease, asthma, stroke and protecting the brain (Akpuaka 2009). In Antimicrobial screening test on *Mangifera indica*cv. Sein Talone leaf extracts, the ethanol extract showed the highest inhibiting zone on *Bacillus subtilis* (27.09 mm), followed on *Agrobacterium tumefaciens* (22.06 mm), *Candida albicans* (19.85 mm), *Saccharomyces cerevisiae*(19.67 mm) and *Bacillus pumilus* (18.83 mm). Methanol extract only effects on *A. tumefaciens* (18.48 mm). Acetone extract effects on *A. tumefaciens* (17.14 mm) and *B. pumilus* (16.90 mm). Ethyl

acetate extract only effect on *A. tumefaciens* (15.59mm).Water extract also only on *A. tumefaciens* (13.39 mm).

Petroleum ether extract has no zone of inhibition on all the test organisms. This indicates that the activity of the extract is influenced by the solvent used for extraction. The test organism *Bacillus subtilis* (IFO 90571) causes the fever. *A. tumefaciens* (IFO 5431) is a plant pathogenic bacterium that causes the plant diseases. *Candida albicans* (NITE 0954-2) is a pathogenic yest, causes the candidiasis. *S. cerevisiae* (NITE 52847) provide food spoilage. *B. pumilus* (IFO 905771) happens the wound and burn infection. This proves that *M. indica* cv. Sein Talone leaves could be used in the treatment of infection caused by such pathogens as *B. subtilis, A. tumefaciens, C. albicans, S. cerevisiae* and *B. pumilis.* The test organisms *Escherichia coli, Pseudomonas fluorescens* and Staphylococcus aureus have no effect on any extracts. A further study of the test organisms and extracts is in progress to isolate, characterize and elucidate the structure of the bioactive compounds present which were responsible for potent pharmacological activity.

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