WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 9, 703-714 Research Article ISSN 2278 – 4357

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF AQUEOUS SEED EXTRACTS OF MACROTYLOMA UNIFLORUM

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Article Received on
27 June 2018,

Revised on 17 July 2018,
Accepted on 07 August 2018

DOI: 10.20959/wjpps20189-11953

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ABSTRACT

The present study was carried out to understand the antimicrobial activity of aqueous extracts. The antibacterial activity of *Macrotyloma uniflorum* (Horse gram) was evaluated against ten bacterial strains including Gram-positive (*S.aureus and K.pneumoniae*) and Gram-negative bacteria (*E. coli*) and fungal strains used were (*A. flavus* and *Verticillum*) by agar-cup diffusion method. Aqueous seed extract showed the best results against all the bacteria and fungal stains and showed maximum zone of inhibition. Phytochemical investigation of aqueous extracts showed the presence of saponins, alkaloids and flavonoids as major phytoconstituents.

KEYWORDS: *Macrotyloma uniflorum*, Phytochemical, bacterial and fungal stains.

INTRODUCTION

Plants have been utilized as medicines for thousands of years. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations. Eventually information regarding medicinal plants was recorded in herbals. Drug discovery from medicinal plant led to the isolation of early drugs such as cocaine, digitoxin and quinine, in addition to morphine, of which some are still in use.[1]

Medicinal plants are used as herbal remedies for human disease.[2] Plants have a great potential for manufacturing new drugs of great potential for producing new drugs of great advantage to mankind. They are many approaches to search for principles in biologically active plant.[3] The consent of traditional medicine are different from health care and the enhancement of microbial resistance to sustaining antibiotics lead researchers to scrutinize the antimicrobial compound.[4]

Phytochemicals are bioactive compounds of plants that work withdietary fiber nutrients and to protect against various diseases. They aremay be non-nutritive compounds. These phytochemicals are commonly known as secondary metabolites that present very small mass in higher plants and they include the flavonoids, terpenoids, alkaloids, tannins, steroids and many others compounds.[5]

In view of increasing the resistance to prevailing antimicrobial agents and herbal drugs are investigated as very significant source for the finding of new agents/compounds for treating various infirmity related to infections of bacteria.[6]

The plant *Macrotyloma uniflorum* seed is a highly nourishing food legume rich in protein, calcium, iron and polyphones. Horse grams that fail to meet food grade standard can be utilized as livestock feed, because of their high protein content and lack of digestive inhibitors.[7] Present work was target to study the photochemical analysis of seed extracts of *M. uniflorum* also to check the antimicrobial activity against some human fungal and bacterial strains.

MATERIALS AND METHODS

Collection of sample

The ***Macrotyloma uniflorum*** plant seed collected from Kolli Hills in the Namakkal District, Tamil Nadu, India. Historically, plants are utilized as a folk medicine against various type of diseases.

Horse gram seed

Processing of plant sample

The horse gram seeds samples were dried under shade, then make it fine powder form (80 mesh sieve size) by electrical grinder. Powdered sample stored in clean paper bags. And then 10g of dried powder samples were used for the preparation of aqueous extract.

Preparation of aqueous extract of plant sample

The collected samples were shade dried at room temperature and then ground to a fine powder in a mechanic grinder. The 20g of powdered material was then extracted using 200ml of aqueous solvent extraction in the ratio 1:10 using Soxhlet apparatus. After extracting solvent were removed by evaporating on water bath which give a solid mass of the extract.

Phytochemical test

Test for Alkaloids

1. **Mayer' s test**

To a sample extract, add two drops of Mayer‟s reagent and mixed well. The white creamy precipitate confirms the positive result of alkaloids.

Tests for Flavonoids

Lead acetate test

1. Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates that the presence of flavonoids.
2. **H_2SO_4 test:** Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates that the presence of flavonoids.

Test for Phenolics compounds

1. **Ferric chloride test:** The extract is treated with 2ml of water and 10% aqueous ferric chloride solution. The Blue or green coloration is observed.
2. **Gelatin test**: About 1% solution of gelatin containing 10% NaCl is added to the ethanolic extract. The white precipitate formation is observed.

Tests for Terpenoids

Salkowski s Test

5 mg of the extract of the leaves, flowers and seeds was mixed with two ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. An appearance of reddish brown colour in the inner face was indicates that the presence of terpenoids.

Tests for Saponins

The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins.

Test for Tannins

1. **Lead acetate test:** The Ethanolic extract is treated with few drops of 1% lead acetate solution. The Yellow or red precipitate formation is observed.
2. **Ferric chloride test**: The Ethanolic extract is treated with 2ml of $FeCl_3$ solution. The Blue or Black precipitate formation is observed.

Test for Glycosides

Glycoside test: 0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

Strains used

The Antimicrobial activity of *Macrotyloma uniflorum* extracts was studied against bacterial strains viz. *E. coli* (Gram-negative), *S.aureus* (Gram positive) and *K.pneumoniae* (Gram-positive). The fungal strains used were *A. flavus* and *Verticillum*.

Composition of Media

The medium should be such that it will promote aster growth of microorganisms. (Refer Table No. a & b).

Table a: Composition of Mueller-Hinton agar media (for bacterial activity).

Ingredient	Quantity
Peptone	300g
Sodium chloride	17.5
Yeast extract	1.5
Agar	2.0 g
Distilled water	100ml

Preparation of Antibacterial agar

1. Suspend 28 g of medium (or the components noted above) in 1 liter of purified water.
2. Mix thoroughly.
3. Heat with constant agitation and boil for 1 minute to until completely dissolve the components.
4. Autoclave at 121°C for 15 minutes.
5. Cool to 45°C.
6. Pour cooled Mueller Hinton Agar into sterile petri dishes on a level, horizontal surface to give uniform depth.

Note: The plates should be spilled to a depth of 4 mm (approximately 25 ml of liquid agar for 100-mm plates and a for 150-mm plates 60 ml of liquid agar, but in any case to a deliberated depth of 4 mm). Plates that are too shallow will fabricate false persuadable results as the antimicrobial compound will diffuse further than it should, generating larger zones of inhibition. Inversely, plates poured to a depth >4 mm will result in false impervious to results.

7. Allow to solidify at room temperature.
8. Check prepared Mueller Hinton Agar to ensure the final pH is 7.3 ±1 at 25°C.

Note: If the pH is <7.2 certain drugs will appear to lose potency (aminoglycosides, quinolones, macrolides), while other agents may appear to have excessive activity (tetracycline). If the pH is >7.4, the opposite results may occur.

9. Prepared media can be stored at 4 to 8°C. Mueller-Hinton agar is stable for approximately 70 days from the date of preparation.

Proper drug of tetracyclin controls were used indicate the zone of inhibition of the control and the sample. Sample was taken at concentration of 100 µl for testing antibacterial activity. The sample diffused into the medium fabricate a concentration gradient. After the incubation period, the measured zones of inhibition were represent in mm. The tabulated results represent the actual readings control.

Table b: Composition of rose-bengal Agar Media (For Antifungal activity).

Ingredient	Quantity
Dextrose	15.0gm
Magnesium Sulfate	0.5gm
Chloramphenicol	0.1gm
Rose Bengal	0.05gm
Agar	15.0gm

Preparation of Antifungal agar

1. Suspend 32.15 g of medium (or the components noted above) in 1 liter of purified water.
2. Mix thoroughly.
3. Heat with constant agitation and boil for 1 minute to until completely dissolve the components.
4. Autoclave at 121°C for 15 minutes.
5. Cool to 45°C
6. Pour cooled rose Bengal agar into sterile petri dishes on a level, horizontal surface to
7. Give uniform depth.

Note: The plates should be spilled to a depth of 4 mm (approximately 25 ml of liquid agar for 100-mm plates and a for 150-mm plates 60 ml of liquid agar, but in any case to a deliberate depth of 4 mm). Plates that are too shallow will fabricate false persuadable results as the antimicrobial compound will diffuse further than it should, generating large zones of inhibition. Inversely, plates poured to a depth >4 mm will result in false impervious to results.

8. Allow to solidify at room temperature.
9. Check prepared Mueller Hinton Agar to ensure the final pH is 7.3 ±1 at 25°C.

Note: If the pH is <7.2 certain drugs will appear to lose potency (aminoglycosides, quinolones, macrolides), while other agents may appear to have excessive activity (Fluconazole). If the pH is >7.4, the opposite results may occur.

10. Prepared media can be stored at 4 to 8°C. Rose Bengal agar is stable for approximately 70 days from the date of preparation.

Proper drug of Fluconazole controls were used to indicate the zone of inhibition of the control and the sample. Sample was taken at concentration of 100µl for testing antifungal

activity. The sample diffused into the medium fabricate a concentration gradient. After the incubation period, the measured zones of inhibition represent in mm. The tabulated results represent the actual readings control.

RESULTS AND DISCUSSION

Phytochemical screening of secondary metabolites.

S.no	Parameter	Aqueous Extract of *Macrotyloma uniflourm*
1.	Alkaloids	++
2.	Flavonoids	++
3.	Saponins	++
4.	Phenols	+
5.	Glycosides	+
6.	Tannins	+
7.	Terpenoids	+

(+) indicates the presence of phytocompound **(-)** indicates the absence of phytocompound and **(++)** indicate the highly presence.

The aqueous extract shows the presence of alkaloids, flavonoids, saponins, tannins, glycoside, and terpenoids *Macrotyloma uniflourm* seed extracts Aqueous were subjected to phytochemical screening for secondary metabolites.

This result of our present study is further supported with similar studies reported by Sofowra,*et al*, 1993.

Antibacterial activity

The antibacterial assay was performed by using the agar diffusion and the pour plate method. Using Erythromycin as standard the antibacterial activity of aqueous plant extract of *Macrotyloma uniflorum* by pour plate method against *E.coli, S.aureus* and *K.pneumoniae* strains are presented in the table:1.

And, the one another table: 2 shows antibacterial activity of aqueous plant extract of *Macrotyloma uniflorum* by cup plate method against *E.coli, S.aureus* and *K .pneumoniae.*

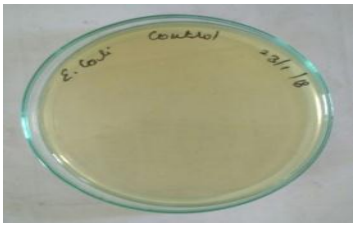
Table 1: Antibacterial activity of aqueous plant extract of *Macrotyloma uniflorum* by pour plate method.

S.No	Name of Bacteria	Gram	Bacteria growth in control plate	Bacterial growth in test plate
1	*E.coli*	-ve	+	_
2	*S.aureus*	-ve	+	_
3	*K.pneumoniae*	-ve	+	_

Table 2: Antibacterial activity of aqueous plant extract of *Macrotyloma uniflorum* by cup diffusion method.

Extract	Organism (Bacteria)	Volume of Sample (Ml)	Zone of Inhibition (Cup Diffusion Methods)(Mm)
Aqueous extract	*E.coli*	40 µl	0.9
		60 µl	3
		80 µl	5
		100 µl	7
	S.aureus	40 µl	2
		60 µl	4
		80 µl	5
		100 µl	5
	K.pneumoniae	40 µl	3
		60 µl	4
		80 µl	5
		100 µl	6

CONTROL



POUR PLATE **CUP PLATE**

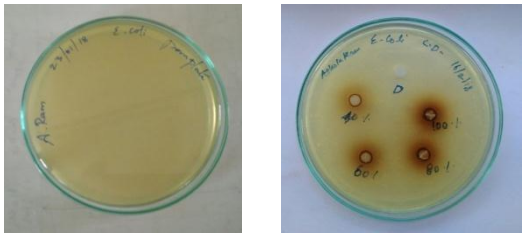
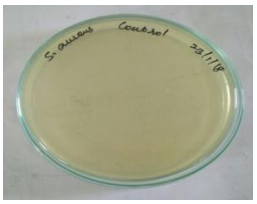


Figure 1: Antibacterial activity of *Macrotyloma uniflorum* by cup diffusion method: *Escherichia coli.*

CONTROL



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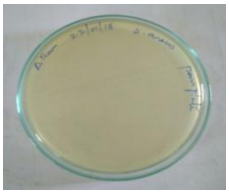
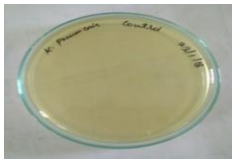
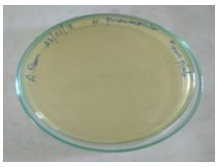


Figure 2: Antibacterial activity of *Macrotyloma uniflorum by cup diffusion method: Staphylococcus aureus.*

CONTROL



POUR PLATE **CUP PLATE**



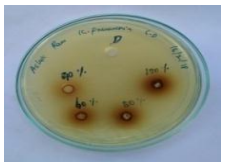


Figure: 3 Antibacterial activity of *Macrotylomauniflorum by cup diffusion method: Klepsiell apneumoniae.*

Antifungal screening

Antifungal activity was studied by using Rose Bengal Agar medium by Cup Diffusion Method using Fluconazole standard against the fungal strains.

The results of the antifungal screening by pour plate method using different fungal strains namely *Aspergillus flavus and Verticillum* are shown below. The aqueous extract of *Macrotyloma uniflourm* seed was used to screen for its antifungal effect by the above method.

Table 3: Antifungal screening of *Macrotyloma uniflourm* seed by cup diffusion method.

Name of the Microorganism	Zone of inhibition (mm) *Macrotyloma uniflourm*				
	Standard Fluconazole 60(µg)	40(µg)	60(µg)	80(µg)	100(µg)
Aspergillus flavus	2.5	0.8	1.0	1.3	1.5
Verticillium	2.0	0.7	1.0	1.2	1.4

The zone of inhibition developed by *Macrotyloma uniflorum* against the selected fungal strains reveals that the *Macrotyloma uniflorum* exhibit effective of antifungal activity.

The zone of inhibition developed by the *Aspergillus flavus* has the higher antifungal activity when compared to the *Verticillum.*

Pour plate method

Table 4: Antifungal activity of aqueous seed extract of *Macrotyloma uniflorum* by pour plate method.

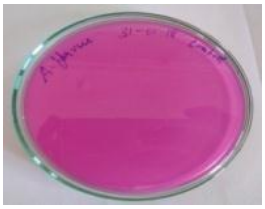
S.No	*Name of the Microorganism*	Bacterial growth on control plate	Bacterial growth on test plate with plant extract
1	*Aspergillus flavus*	+	-
2	*Verticillum*	+	-

(+) indicates growth of organism (-) indicates no growth organisms

The table results obtained clearly states that the sample posses 100% antifungal effect against the selected fungal strains.

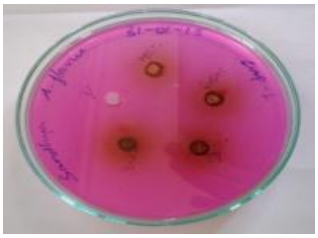
The absence of fungal growth signifies the plant sources posses effective antifungal activity against selected infectious fungal strains. The results of our present study in further supported with the similar reports presented by.[8]

CONTROL



Aspercillus flavus

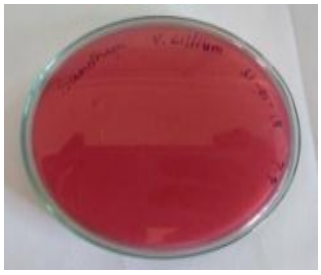
POUR PLATE method



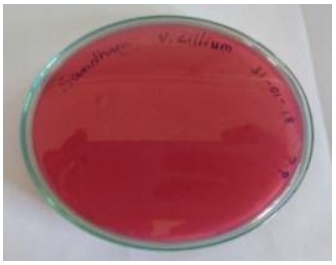
CUP DIFFUSION method

Fig 4: Antifungal activity of *macrotyloma uniflorum* by cup diffusion method Aqueous Extract.

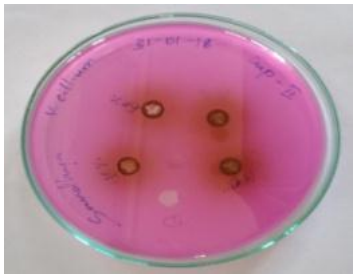
CONTROL



Verticillum



POUR PLATE method



CUP DIFFUSION method

Fig 5: Antifungal activity of *macrotyloma uniflorum* by cup diffusion method Aqueous Extract.

CONCLUSION

Results of the study showed that the aqueous extract of *Macrotyloma uniflorum* seed showed significant antimicrobial activity (antibacterial and antifungal) when compared with the standard drugs. Further, phytochemical investigation of these extracts showed the presence of alkaloids, saponins and flavonoids as major constituents in aqueous extract respectively. So it can be concluded that flavonoids can be used in treating various skin diseases. This study can be further extended to isolate major constituents from these extracts which are responsible for treating the skin diseases and also formulation of these isolated compounds into a proper external dosage form for treating various skin diseases.

ACKNOWLEDGEMENT

The authors are thanks to Department of Biochemistry, Periyar University, Salem, TamilNadu for providing us well furnished lab and equipments.

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