



PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF SEED EXTRACTS OF *MACROTYLOMA UNIFLORUM* (HORSE GRAM)

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ABSTRACT

The present study deals with preliminary phytochemical screening of seed extracts of Macrotyloma uniflorum (horse gram) and its antimicrobial activity against human bacterial pathogens. Aqueous and alcoholic extracts of Seeds of horse gram were evaluated for antibacterial using well diffusion method against some human bacterial pathogens. Phytochemical studies revealed the presence of carbohydrate, steroid, tannins, phenol, protein and amino acid, and absence of alkaloids, glycosides, flavonoids and saponins. Antibacterial activity was tested against 9 human bacterial pathogens using alcoholic and aqueous extract. On comparing the antibacterial activity by alcoholic and aqueous extracts, they showed a moderate to high activity against 9 human pathogens. On considering the rate of antimicrobial activity against pathogens, alcoholic extract showed a better result in controlling the growth of bacteria than that of aqueous extract.

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Keywords: *M. uniflorum*, Aqueous, Alcoholic extracts, Phytoconstituents, Antibacterial activity.

Contribution/ Originality

The paper's primary contribution is finding the phytochemical analysis and antibacterial activity of the seed of horse gram plant *M.uniflorum*. The overall results showed promising baseline information for the potential therapeutic use of seeds *M.uniflorum* against human bacterial pathogens.

1. INTRODUCTION

Horse Gram, *M. uniflorum* is the most extensively grown pulse in south India, the maximum area being in Andhra Pradesh, Karnataka and Tamil Nadu.

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Macrotyloma is a nutritious food legume. It is rich in protein, iron, calcium and polyphenols. Green plant of horse gram is a valuable green manure. Horse grams that fail to meet food grade standard can be used as livestock feed, because of their high protein content and lack of digestive inhibitors [1]. It has the greatest potential for the utilization of nutraceuticals, forage and food for malnourished and drought prone areas of the world [2]. The presence of flavonoids, terpenoids, glycosides, tannins, steroids, and saponins etc has an impact on anti-bacterial, anti-fungal and anti-oxidant activity [3]. Characterization and isolation of kaempferol -3-O- B-D-glycoside, B-sitosterol and stigmasterol in *M. uniflorum* [4] and cytotoxicity assessment of this plant [5] were reported.

It is famous for its medicinal use because different parts of the plants are used for the treatment of heart disease, asthma, bronchitis, urinary discharges and for treatment of kidney stone [6]. Horse gram water is prescribed for treating jaundice in Andhra Pradesh [7]. Horse gram helps in lowering cholesterol levels and plays a role in antioxidation [8]. Present work was aimed to study the photochemical analysis of seed extracts of *M. uniflorum* and also to screen the antibacterial activity against some human bacterial pathogens.

2. MATERIALS AND METHODS

2.1. Plant Materials

M. uniflorum seeds were washed, shadow dried and subjected to pulverization to get coarse powder.

2.2. Preparation of Aqueous Extract

The aqueous extract was prepared by mixing 10g of powdered seeds of this plant by mixing with sterile distilled water and boiled to slow heat for 2 hours. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and the procedure was repeated twice. The extracted supernatant was concentrated to make the final volume one-fourth of the original volume [9]. It was then autoclaved 121°C at 15 lbs pressure and stored at 40°C.

2.3. Preparation of Alcoholic Extract

The powdered seeds of about 20g were extracted with alcoholic in a soxhlet apparatus. Then, the extract was evaporated in a rotatory vacuum evaporator at 40°C under reduced pressure. The crude extract of about 13g was obtained which is equivalent to about 20% of total extraction.

2.4. Phytochemical Tests

The preliminary phytochemical analysis was performed as per the method [10]. Using standard protocols the biomolecules such as carbohydrate, steroid, tannins, phenol, protein, amino acid, alkaloids, glycosides, flavonoids and saponins were tested for their presence.

2.5. Microorganisms

The bacterial strains of *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas argentinensis*, *Pseudomonas sp*, *Bacillus subtilis*, *Vibrio harveyi*, *Salmonella paratyphi*, *Pseudomonas aeruginosa* and *Vibrio mimicus* were maintained in nutrient agar slants respectively and stored at 4°C.

2.6. Preparation of Stock Culture and Bacterial Suspension

Standard nutrient agar (NA) medium was used for bacterial strains throughout the research. In a hard glass screw cap tube, sterile slants of NA were prepared. Older cultures were transferred to freshly prepared NA slants separately for each species via sterile bacterial loop. In such a way, nine test tubes were freshly prepared for each bacterial pathogen. These test tubes of inoculated slants were incubated at 37⁰ C in an incubator. After 18-24 hours of inoculation each culture was used throughout for antibacterial screening studies. For preservation of the stock culture, one set of culture slants were kept in polythene bag, properly tied and preserved as stock culture at 10⁰ C. Subcultures were maintained and tested at 3 to 4 week intervals to ensure culture viability.

2.7. Antibacterial Activity

The extracts were screened for their antibacterial activity invitro by agar well diffusion method against *E. coli*, *K. pneumonia*, *P. argentinensis*, *Pseudomonas sp*, *B. subtilis*, *V. harveyi*, *S. paratyphi*, *P. aeruginosa* and *V. mimicus*. Bacterial colonies were selected and transferred to 5 ml broth with a loop and the broth cultures were incubated at 37° C for 24 hours. For Screening the antibacterial activity Muller-Hinton agar was prepared and seeded with respective human bacterial pathogens. Then the wells were made by using a sterile cork borer and was added with different volumes (50µl, 75µl and 100µl) of the crude extract of *M. uniflorum* (1g/10ml distilled water) and kept for incubation at 37° C for 24 hours. Triplicates were maintained for the said concentrations. After incubation at 24h and 48h the results were recorded for the formation of zone inhibition. Streptomycin (10µg/5ml) was used as standard [11] to compare the antibacterial activity of the test plant.

2.8. Statistical Analysis

Zone of inhibitions observed in the present study were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test (P<0.05) using Statistics (Statsoft Inc., Tulsa, USA). Values expressed are means of three replicate determinations ± standard deviation [12].

3. RESULTS

3.1. Phytochemical Analysis

Aqueous and alcoholic extract of seeds of *M. uniflorum* were taken and analysed to find the presence of various phytoconstituent (Table 1). The result showed positive respond to the

phytoconstituents like carbohydrate, steroid, tannins, phenol, protein and amino acid. Both extracts of seeds of *M. uniflorum* exhibited negative result for alkaloids, glycosides, flavonoids and saponins.

3.2. Antibacterial- Sensitivity Test

Antibacterial activity of *M. uniflorum* was screened against human bacterial strains such as *E. coli*, *K. pneumonia*, *P. argentinensis*, *Pseudomonas* sp., *V. harveyi*, *S. paratyphi*, *P. aeruginosa*, *V. mimicus* and *B. subtilis* and reported in table 2. Aqueous extract at 100 µl concentration, the antibacterial activity was found good against *S. paratyphi*, *P. argentinensis*, *V. harveyi*, *V. mimicus* and *B. subtilis* by showing zones of inhibition of 17±1.50, 15±1.80, 15±1.00, 14±2.00 and 15±1.73 mm respectively. Antibacterial activity was found to be more pronounced against *S. paratyphi* (17 mm). The aqueous extract was less active against *E. coli* than standard antibiotic (Table 2; Figure 1).

Alcoholic extract of *M. uniflorum* at the concentrations of 50µl, 75µl and 100µl were tested against pathogenic bacteria. Alcoholic extracts at 100µl concentration antibacterial activity showed 17±1.11 mm of inhibition against *E.coli* while the zone of inhibition was 17±1.54, 15±1.00, 14±1.13, 14±1.09 mm respectively against *P. argentinensis*, *Pseudomonas* sp., *S. paratyphi* and *V. mimicus* and was 13±1.52 mm zone of inhibition against *P. aeruginosa* and 12±1.0 mm zone of inhibition against *K. pneumonia*. However, *B. subtilis* and *V. harveyi* bacteria did not show any zone of inhibition against alcoholic extract (Table 3; Figure 2).

When comparing the antibacterial activity by using one way anova multiple test, the obtained results were not significant to one another ($P>0.05$). *M. uniflorum* extracted with aqueous and alcoholic extracts, has more or less similar impact over human bacterial pathogens. Both the extracts showed a similar activity on *V. mimicus*, *K. pneumonia*, *P. argentinensis* and *P. aeruginosa*, whereas alcoholic extract showed a better antibacterial activity against *S. paratyphi* and *E. coli*. Study on gram positive bacteria, *B. subtilis*, revealed that alcoholic extract exhibited no zone of inhibition whereas aqueous extracted sample exhibited a zone of inhibition (15 mm).

4. DISCUSSION

In the present investigation, phytochemical screening of seeds of *M. uniflorum* extracted with aqueous and alcoholic solvents and the antibacterial efficacy of the extracts was evaluated on the basis of zone of inhibition. The phytochemical analysis revealed that *M. uniflorum* contains bioactive substances which reflected on the antibacterial properties of the plant against human bacterial pathogens. Presences of bioactive compounds from plant extracts with various solvents were reported [13-19].

Among the two extracts studied the alcoholic extract was found to have better antibacterial activity than the seeds extracted with aqueous. Organic extracts provided more potent antibacterial activity as compared to aqueous extracts. The polarity of secondary metabolites and antibacterial compounds make them more readily extracted by organic solvents because Secondary metabolites

are more soluble in organic solvents than water, and using organic solvents does not negatively affect their bioactivity against bacterial species suggesting that organic solvents are clearly better solvents of antimicrobial agents [20, 21]. Antibacterial activity exhibited by the seed extracts of *M. uniflorum* may be due to the better solubility of the active compounds in solvent [22]. The antimicrobial efficiency exhibited against gram positive and gram negative bacterial human pathogens might be due to the presence of phytoconstituents such as phenol and tannins. Similar results were reported against bacterial human pathogens due to phytochemicals obtained after extraction with various solvents in *M. uniflorum* [23, 24] and also in *Hyptis suaveolens*, *Aerva lanata* and *Andrographis paniculata* respectively [25, 26] and Usha Raja Nanthini, et al. [27].

The findings of the present study suggested that seeds of *M. uniflorum* have potent antibacterial activity against human bacterial pathogens. The presence of tannins and phenolic compounds noticed in the present study are responsible for antibacterial activity and they may bring out antibacterial efficiency by cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhesions as suggested in earlier study [28].

5. CONCLUSION

Extracts of *M. uniflorum* demonstrated a broad –spectrum of activity against human bacterial pathogens. Based on the phytochemical analysis, the seeds of *M. uniflorum* contain more bioactive compounds which in turn elicit the antimicrobial efficiency of the plant against the tested human bacterial pathogens of the present study. Further studies on phytochemical characterization on the seeds of *M. uniflorum* may throw light on the specific component responsible for such antibacterial activity.

Table-1. Preliminary photochemical screening of *Macrotyloma uniflorum*

S.NO	Name of Phytoconstituents	Aqueous Extract	Alcoholic Extract
1	Carbohydrate	+	+
2	Steroids	+	+
3	Tannins	+	+
4	Protein	+	+
5	Phenolic	+	+
6	Amino acid	+	+
7	Alkaloids	-	-
8	Glycosides	-	-
9	Flavonoids	-	-
10	Saponins	-	-

+ Presence –Absence

Table- 2. The antibacterial activity of aqueous extracts of the plant *Macrotyloma uniflorum*

S.No	Bacterial culture	Zone Of Inhibition(mm)				
		DMSO Control	Standard Streptomycin (5mg/ml)	50µl	75µl	100µl
1	<i>V. mimicus</i>	C	33±2.00 ^a	11±1.00 ^b	14±1.40 ^b	14±2.00 ^b
2	<i>E. coli</i>	C	21± 2.00 ^a	10±1.00 ^b	11±1.73 ^{bc}	13±1.32 ^c
3	<i>K. pneumonia</i>	C	33±2.00 ^a	12±1.20 ^c	13±0.57 ^b	14±1.00 ^b
4	<i>P. argentinensis</i>	C	25±2.64 ^a	12±1.00 ^b	13±1.00 ^b	15±1.80 ^b
5	<i>Pseudomonas sp</i>	C	30±1.73 ^a	11±1.50 ^b	11±1.00 ^b	13±1.32 ^b
6	<i>B. subtilis</i>	C	23±1.32 ^a	11±1.50 ^c	12±1.00 ^c	15±1.73 ^b
7	<i>V. harveyi</i>	C	22±1.52 ^a	12±1.00 ^b	14±1.25 ^b	15±1.00 ^b
8	<i>S. paratyphi</i>	C	21±1.00 ^a	15±1.00 ^c	16±1.32 ^c	17±1.50 ^b
9	<i>P. aeruginosa</i>	C	32±2.00 ^a	10±1.00 ^c	12±2.00 ^{ab}	14±1.00 ^b

Mean within a column followed by the same letters ^(a,b,c) are significantly different according to one way ANOVA and Duncan's multiple range test ($P < 0.05$).

Table-3. The antibacterial activity of alcoholic extracts of the plant *Macrotyloma uniflorum*

S.No	Bacterial culture	Zone Of Inhibition(mm)				
		DMSO Control	Standard Streptomycin (5mg/ml)	50µl	75µl	100µl
1	<i>V. mimicus</i>	C	32±1.41 ^a	10±0.80 ^c	12±0.96 ^c	14±1.09 ^b
2	<i>E. coli</i>	C	32±1.73 ^a	13±1.11 ^c	15±1.34 ^b	17±1.54 ^b
3	<i>K. pneumonia</i>	C	33±1.52 ^a	10±0.50 ^c	11±0.57 ^b	12±1.0 ^b
4	<i>P. argentinensis</i>	C	30±1.25 ^a	10±0.50 ^c	15±1.00 ^b	17±1.11 ^b
5	<i>Pseudomonas sp.</i> ,	C	33±1.25 ^a	10±0.56 ^c	12±0.82 ^b	15±1.00 ^b
6	<i>B. subtilis</i>	C	26±1.64 ^a	-	-	-
7	<i>V. harveyi</i>	C	31±1.87 ^a	-	-	-
8	<i>S. paratyphi</i>	C	30±1.32 ^a	10±0.05 ^a	11±0.59 ^a	14±1.13 ^b
9	<i>P. aeruginosa</i>	C	32±1.50 ^a	10±1.12 ^c	11±1.40 ^{bc}	13±1.52 ^b

Mean within a column followed by the same letters ^(a,b,c) are significantly different according to one way ANOVA and Duncan's multiple range test ($P < 0.05$).

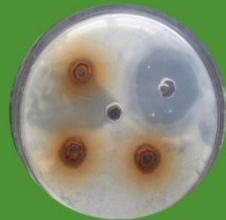
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Fig 2. Shows the antibacterial activity of aqueous extracts of the plant *Macrotyloma uniflorum*



Vibrio mimicus



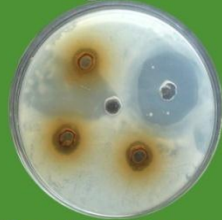
Escherichia coli



Klebsiella pneumonia



Pseudomonas argentinensis



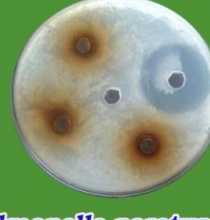
Pseudomonas sp.



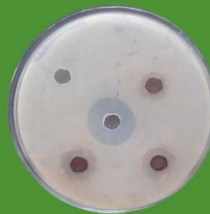
Bacillus subtilis



Vibrio harveyi



Salmonella paratyphi



Pseudomonas aeruginosa

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