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Research Article

STUDY OF ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF DIFFERENT PARTS OF MOMORADICA MONADELPHA (TILKOR): AN ANCIENT INDIAN TRADITIONAL MEDICINAL PLANT

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A B S T R A C T

Plants have been a valuable source of natural products since the time immemorial. The use of plant and plant products for pharmaceutical purposes has gradually increased in India. The use of plant extract with antimicrobial properties can be of great significance in therapeutic treatments. The present work is carried out on different parts of Momoradica Monadelpha (Tilkor) on the basis of the literature obtained from the ethno medicinal documentation. Keeping in view the tremendous ethno medicinal use of Momoradica Monadelpha the study was aimed to scientifically validate antibacterial property of different plant components (leaf, stem & root) of Momoradica Monadelpha.

INTRODUCTION

The effect of plant extracts on bacteria and fungi have been studied by a very large number of researchers in different parts of the world as well as in India. Tilkor is an important rejuvenating drug used in Ayurveda. The whole plants viz root, leaves, flowers, seed etc. are used for medicinal purposes. Plant is used in Ayurvedic, Unani, Siddha and Homoeopathy Systems (Guhabakshi *et al.,* 1999)[1]. Apart from above, *Momoradica Monadelpha* also possess marked antimicrobial properties viz- antibacterial properties and antifungal properties (Agrawal *et. al*., 2004)[2]. All the properties have made this plant very important in the treatment of human and plant diseases. Thus the main objective of this study was to investigate the antibacterial activity of different plant components (leaf, stem and root) of *Momoradica Monadelpha,* which are used in traditional medicinal system of Kumaun Himalaya for the treatment of diarrhoea, urinary tract infection, typhoid etc.

MATERIALS AND METHODS

***Collection of Plant material and pre-extraction preparation*:** The plant *Momoradica Monadelpha* was collected from the medicinal plant garden of Shri Himanshu Shekhar Malik (Jale). Prof. Shashi Shekhar Narayan Sinha, Darbhanga the botanical identity of plant was established and authenticated from the different parts of the plant viz- leaf, stem and root were separated cut and air dried in shade at laboratory temperature.

***Preparation of methanolic extract of: Momoradica Monadelpha*:** The plant extract of *Momoradica Monadelpha* was prepared by standard methodology (Pharmacopoeia of India, 1985)[3].

Extraction of plant component- leaf: The plant leaf extract was prepared by soaking 60 g shade dried powdered leaf material of *Momoradica Monadelpha* in 60% ethyl alcohol for 25 hr with intermittent stirring at 50^0C with the help of magnetic stirrers. The infusions were filtered through muslin cloth to get the supernatant. The filtrate was dried under reduced pressure with the help of rotary vacuum pump evaporator to get the final extract. The percent yield of the leaf extract was recorded approximately 15 percent.

Extraction of plant component– stem: The plant stem extract was prepared by soaking 60 g shade dried stem powdered material in 60% of ethyl alcohol for 25 hr with intermittent stirring at 50^0C with the help of magnetic stirrers. The infusions were filtered through muslin cloth to get the supernatant. The filtrate was dried under reduced pressure with the help of rotary vacuum pump evaporator to get the final extract. The percent yield of the stem extract was recorded approximately 8 % percent.

Extraction of plant component- root: The plant root extract was prepared by soaking 60 g shade dried root powdered material in 60% of methyl alcohol for 25 hr with intermittent stirring at 50^0C with the help of magnetic stirrers. The

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infusions were filtered through muslin cloth to get the supernatant. The filtrate was dried under reduced pressure with the help of rotary vacuum pump evaporator to get the final extract. The percent yield of the root extract was approximately 7% percent. The shade dried powdered aqueous extract of leaf, stem & root of the plant was used throughout the study. This undiluted plant extract was diluted, wherever required, in N.S.S. (Normal Saline Solution). The standard procedure suggested by Cruiskshank *et al.* (1975)[4].

Preparation of Microbial Strains

Text- Bacteria

The antibacterial activity of different plant component (leaf, stem and root) was assessed against three bacterial species *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* and they were procured from the Department of Microbiology, Darbhanga Medical College, Laheriasarai, Darbhanga. The Inoculums for the study were prepared by growing these bacterial species in nutrient agar at 33^0C for 15 hr & purity of culture was checked after 9 h of incubation, kept at 5 ^{0}C until used. The bacterial cultures were diluted in sterile N.N.S. for further study via serial two fold dilution (Cruiskshank *et al.,* 1975)[4].

Table 3.1 Bacterial dilutions used in the study

Bacterial Strains	Dilutions				
Escherichia Coli	1:10	1:100	1:1000	1:10000	1:100000
Staphylococus aureus	1:10	1:100	1:1000	1:10000	1:100000
Salmonella typhi	1:10	1:100	1:1000	1:10000	1:100000

Antibiogram study: Agar Disk diffusion Techniques

Filter paper disc impregnated with different plant component viz- leaf, stem and root of *Momoradica Monadelpha* used to study the antibacterial activity (reduction in colony diameter).

Antibacterial Assay

Antibacterial activity of different plant component (Leaf, stem and root) of different concentration was determined by the method of **Murray *et al.*** (1995)[5]. Efficacy of the extract was determined by comparing the zone of inhibition around the sensitivity disc. The bacterial suspension (each organism) of different concentrations (1:10,1:100,1:1000,1:10,000, 1:10,0000) were spread over the plates containing nutrient agar (Hi-Media) using a sterile cotton swab in order to get uniform microbial growth on test plates. Under aseptic conditions, empty sterilized filter paper disc (Hi-Media, 10 diameter.) were impregnated with different plant component extracts (leaf, stem, and root) of different concentrations (1:3, 1:9, 1:2:7, and 1:36) and dried at room temperature Sterile forceps were used to place each of the discs (loaded with different plant component of different concentration) on agar surface. Paper disc moistened with N.S.S. were placed on a seeded Petri dishes as a control. All the Petri dishes were left for 35 minutes at room temperature to allow the diffusion of plant extract and then they were incubated at 38^0C for 25 hours. The zone of inhibition was measured in mm with a "Hi Antibiotic Zone Scale". Studies were performed in triplicate and mean value was calculated. The results were expressed as mean ± SEM (standard error of mean).

Minimum Inhibitory Concertration (Mic Assay)

Based on the previous study, Minimum Inhibitory Concentration (MIC) of the positive extracts were determined by the method (test with different gradient concentration) suggested by Scott (1989). By this method, the test organisms were seeded uniformly over an agar surface and exposed to decreasing concentrations (from 300 mg/ml to 12.7 mg/ml) of different plant component extracts (leaf, stem and root) diffusing from a paper disc (Disc Diffusion Test). The plates were then incubated at 38 ^{0}C for 28 hours. The bacteria which were sensitive to the plant component they were inhibited from growing in a circular zone around the paper disc.

RESULTS AND DISCUSSION

The antibacterial activity of different plant components (leaf, stem and root) of *Momoradica Monadelpha* aqueous extract against *Salmonella typhi, Staphylococcus aureus* and *Escherichia coli* were summarized in table- 5.1, 5.2 and 5.3. The result of this investigation revealed that the different plant components of *Momoradica Monadelpha* showed antibacterial activity with varying magnitudes. The zone of inhibition (reduction in colony diameter) above 20 mm in diameter was taken as positive result. Generally, most of the test organisms were sensitive to different plant components (leaf, stem and root) extracts of *Momoradica Monadelpha.* Sensitivity of bacterial species against active extract was observed in the decreasing order of *Salmonella typhi>Staphylococcus aureus >Escherichia coli.* It is also evident from the data that all the plant part marked inhibitory effect against two test organism except *Escherichia coli.* The plant part which exhibited highest bacterial activity was Root> Leaf> Stem. There was no zone of inhibition with the control (NSS disc).

Table-5.1: Mean inhibition zone diameter (ZD) by loaded disc (leaf) with 5 mg extract dissolved in NSS against different bacterial strains

Different concentration of plant component leaf (mg/ml)	Bacterial strains											
	Salmonella typhi			*Staphylococcus aureus*			*Escherichia coli*			Control (NSS)		
	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM
400	A	25 + 0.573		A	22 + 0.472		N	-		N		-
200	A	24 + 2.42		A	23 + 0.815		N	-		N		-
100	A	18 + 0.470		A	14 + 0.944		N	-		N		-
50	A	15 + 1.248		A	13 + 0.942		N	-		N		-
25	A	13 + 0.473		N	15 + 0.473		N	-		N		-

Table 5.2 Mean Inhibition zone diameter (ZD) by loaded disc (stem) with 5mg extract dissolved in NSS against different bacterial strains

Different oncentration of plant component leaf (mg/ml)	Bacterial Strains											
	Salmonella typhi			Staphylococcus aureus			Escherichia coli			Control (NSS)		
	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM
400	A	24+0.941		A	23+0.433		N	-		N	-	
200	A	22+1.248		A	18+0.472		N	-		N	-	
100	A	15+0.944		A	13+2.45		N	-		N	-	
50	A	12+1.41		A	10+0.946		N	-		N	-	
25	A	11+1.630		A	5+0.471		N	-		N	-	

Table-5.3: Mean inhibition zone diameter (ZD) by loaded disc (root) with 5 mg extract dissolved in NSS against different bacterial strains.

Different concentration of plant component leaf (mg/ml)	Bacterial strains											
	Salmonella typhi			*Staphylococcus aureus*			*Escherichia coli*			*Control (NSS)*		
	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM
400	A	25 + 0		A	25 + 0.432		N	-		N	-	
200	A	23 + 0.815		A	25 + 0.473		N	-		N	-	
100	A	17 +1.634		A	15 + 2.45		N	-		N	-	
50	A	12 + 1.413		A	16 + 0.942		N	-		N	-	
25	A	14 + 0.815		N	13 +0.475		N	-		N	-	

Key to tables – 5.1, 5.2 and 5.3

Symbol	Meaning
A -	Active
	Not
N -	Active
SEM -	Standard error of the mean
ZD -	Zone diameter(mm)
(-) -	No effect

Studies have been carried out to test antibacterial activity of *B. diffusa* (Abo and Ashide, 1999).[7] Similarly Kumar *et al. (1997)*[8] have also reported antibacterial activity in the Seeds of *B. diffusa* against *Bacillus Subtilis* (zone diameter 3.30mm/mg) *Pseudomonas cichorii* (Zone diameter 6.60 mm/mg) except *Escherichia coli.* Nair and Chanda (2006)[9] reported antibacterial activity of *B. diffusa* leaves against *Pseudomonas aeruginos,* Aladesanmi *et al.* (2007)[10] also reported antibacterial activity of plant *B. difJusa* against *Bacillus subtilis* (Inhibition zone 2.0 mm (40mg/ml) and 6.0 mm (225 mg/ml), *Pseudomonas aeruginosa* (inhibition zone 3 mm (225mg/ml), *Staphylococcus aureus* (inhibition zone 1 mm, 1 mm, 2 mm, 4 mm / 10, 20, 40, 225 mg/ml respectively) except *Escherichia coli.*

However, in the present investigation result obtained showed that two fold dilutions of different plant components viz leaf, stem, and root extracts of *B. diffusa* gives antibacterial activity against *Salmonella typhi, Staphylococcus aureus* except *Escherichia coli*. The Bacteria S. *typhi* causes Typhoid fever, Neonatal meningitis infection via intestinal route (Banavandi 2005, Todar 2005)[11]. The root component of *B. diffusa* showed maximum antibacterial activity against S. *typhi* (inhibition zone 26-12 mm) followed by leaf extract (inhibition zone 25-13 mm) and least in case of stem extract (24 - 11 mm), while the bacteria *Staphylococcus aureus* causes urinary trouble, middle ear infection, scarlet fever, food poisoning, toxic shock syndrome, as well as Gram -ve cocci causing gonorrhoea (Todar 2005). According to result given above root component of *B. diffusa* had maximum antibacterial activity against *S. aureus* (inhibition zone 25-13 mm) followed by leaf component (inhibition zone 23-13 mm) and least in case of stem component (Inhibition zone 23-5 mm).

The result further showed that the root extract possess maximum antibacterial activity against *Salmonella typhi* (Inhibition zone 26 mm) and *Staphylococcus aureus* (Inhibition zone 25 mm) while the stem extract marked least antibacterial activity against *Salmonella typhi* (inhibition zone 24 mm) and *Staphylococcus aureus* (inhibition zone 23 mm) and activity of leaf extract reported against *Salmonella typhi* (inhibition zone 25 mm) and *Staphylococcus aureus* (inhibition zone 23 mm) was intermediate between these two extract (Root and leaf). This appears to be the first study that actually investigated antibacterial activity of different plant component (leaf, Stem and Root) of *B. diffusa* against *S. typhi* and *S. aureus.* However, there have been extensive study on *B. diffusa* seeds and leaf on different bacteria was done by Nair and Chanda (2006); Aladescmi *et al.* (2007) have reported that *B. diffusa* extracts have little activity against bacteria and no antifungal activity against fungi in Nigeria. However, the investigation is much similar to Nair and chanda (2006) as well as Sweta and Verma (2017)[12] in ethanolic extract of plant's components because in all the investigations the plant *B. diffusa* fail to inhibit *E. coli* but as result expressed that plant *B. diffusa* have shown the potential to inhibit the *S. typhi* and *S. aureus.*

Thus result expressed that *B. diffusa* can be used in the treatment of typhoid fever, urinary tract infection, ear infection, fever, food poisoning caused by bacteria *S. typhi* and *S. aureus* respectively. This finding also confirmed that folkloric claims of locally consumed *B. diffusa* extract to cures typhoid fever, ear infection, fever, urinary trouble in local Kumaun Hills. \Minimum Inhibitory Concentration (MIC) for different plant component (leaf, stem and root) extract ranged from 25 mg/ml to 50 mg/ml (table-5.4) as can be seen in the table 5.4 the MIC zone increased with increasing concentration of different plant components. This study also revealed that the root and leaf extract showed maximum activity with MIC value being 27 mg/ml followed by stem extract with MIC value 55 mg/ml. In the present study minimum inhibitory concentration (MIC) for different plant component (leaf, stem and root) extract ranged from 25-55 mg/ml. It is clear from the figure, the zone diameter increased with increasing concentration of different plant components. The results also revealed that the different plant components (leaves, stem and root) of *B. diffusa* were able to inhibit *S. typhi*. Whereas the root and leaf extract was effective against *S. aureus*, while stem extract fail to inhibit said bacterium.

Table 5.4 Minimum Inhibitory Concentration of the active compound against tested bacteria

Plant component	Extract 200 mg/ml	*Salmonella typhi*		*Staphylococcus aureus*		*Escherichia coli*	
		MIC mg/ml	ZD (mm)	MIC mg/ml	ZD (mm)	MIC mg/ml	ZD (mm)
Leaf	NSS	27	12	26	12	-	-
Stem	NSS	55	13	-	-	-	-
Root	NSS	28	14	23	15	-	-

Table 5.5 Relationship among various concentration of different plant component (leaf, stem and root) extracts with zone of inhibition on against *Salmonella typhi* in determination of MIC

Concentration of different plant extract (mg/ml)	Leaf	Stem	Root
	ZD (mm)	ZD (mm)	ZD (mm)
200	23	11	25
100	19	12	15
50	15	13	14
25	12	-	13
12.5	-	-	-

Table 5.6 Relationship among various concentration of different plant component (leaf, stem and root) extracts with zone of inhibition on against *Staphylococcus aureus* in determination of MIC

Concentration of different plant extract (mg/ml)	Leaf	Stem	Root
	ZD (mm)	ZD (mm)	ZD (mm)
400	17	-	18
200	15	-	15

100	12	-	17
50	10	-	16
25	-	-	-

In this study, different bacteria marked different zone inhibition diameter against different plant components showed that root extract have always shown higher zone of inhibition diameter against *S. typhi* with 28 mg/ml (inhibition zone 25 to 13 mm) then stem and leaves extracts. Leaves extract revealed next higher zone of inhibition diameter (inhibition zone 23 to 12 mm) against S. *typhi* where as stem extract marked minimum zone inhibitory diameter (inhibition zone 13 to l1 mm) against the said bacterium.

Present study also revealed zone of inhibition diameter versus plant extracts against *S. aureus.* Root extract have always marked maximum zone diameter (inhibition zone 18 to 15 mm) at higher concentration (400 and 200 mg/ml) where as at lower concentration (500 and 50 mg/ml) leaves revealed higher zone of inhibition (inhibition zone 12 to 10 mm) then stem and root extracts. From above figures it is here by suggested that root extract could be used against diseases caused by *Salmonella typhi* where as root extract and leaf extracts may be also used against diseases caused by *S. aureus* at higher and lower concentration respectively. *B. diffusa* extract is not effective against *Escherichia coli* bacteria.

References

2. Guhabakshi, D.N., P. Sensarma and D.C. Pal (1999). A lexicon of medicinal plants of India (Vol-1). Naya Prakash Culcutta, India.
3. Agarwal, A., S. Srivastava, J.N. Srivastava and M.M. Srivastava (2004). Inhibitory effect of the plant *Momoradica Monadelpha* L. against the dermatophytic fungus *Microsporum fulvum*. J. Environment. Biol. 25 (3): 307-311.
4. Pharmacopoeia of India (1985). Preparation of extracts of herbs. Third edition. Ministry of Health, Govt. of India.
5. Cruiskshank R., J.P. Duguid, B.P. Marmion and R.H. Swain (1975). Medicinal Microbiology,12th Edition Churchill Livingstone. Edinburgh.
6. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenoover and R. H. Yalke (1995). Manual of Clinical Microbiology. 6th Edition ASM, Washington DC.
7. Scott, A.C. (1989). Laboratory control of antimicrobial therapy. In: practical medical Microbiology. (Eds. J.G. Collee, J.P. Duquid, A.G. Fraser and B.P. Marmion) 13th Edn. Churchill Livingetone. Edinburg.
8. Abo, K.A. and J.S. Ashidi (1999). Antimicrobial screening of *Bridelina micrantha, Alchomea cordifolia and Momoradica Monadelpha*. Afr. J. Med. Medical Sci. 28 (3-4): 167-9.
9. Kumar Sushil, G.D. Bagchi and M. P. Darokar (1997). Antibacterial activity observed in the seeds of some Coprophillous plants. *International Journal of Pharmacognosy*. 35 (3): 179-184.
10. Nair, R and S. Chanda (2006). Activity of some medicinal plants against certain pathogenic bacterial Strains. Indian J. Pharmacol. 38 (2): 142-144.
11. Banavandi, M.J.S., M.H. Shahhosseiny, D. Shahbazzadeh, V. Karimi, H. Mirzahoseini, F. Mahboudi, M. Abachi and G. Javadi (2005). Selective Amplification of prt, tyv and inv A genes by Multiplex PCR for rapid detection of *Salmonella typhi. Indian Biomedical Journal*. 9 (3):135-138Todar K. (2005). Salmonellosis. Todar online Text book of Bacteriology.
12. Aladesanmi, A.J., E.O. Iwalewa, A.C. Adebajo, E.O. Akinkunmi, B.J. Taiwo, F.O. Olorunmola and A. Lamikanra (2007). Antimicrobial and antioxidant activities of some Nigerian medicinal plants. Afr. J. of Traditional Complementary and Alternative Medicine. 4 (2): 173-184.
13. Shweta Majgaine , D. L. Verma (2017), Antibacterial Activity of Momoradica Monadelpha L. (Tilkor) On certain Bacteria : *IOSR Journal of Pharmacy* 7 (2) : 1 – 13

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