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SCREENING OF ANTIBACTERIAL ACTIVITY OF FLAVONOID FRACTIONS OF MOMORDICA DIOICA, ROXB.

J. A. Arekar*, A. R. Arekar and G. T. Paratkar
Plant Biotechnology Laboratory, Department of Biotechnology,
GES's N. B. Mehata Science College, Bordi, Dahanu, Dist. Thane. Maharashtra , India.
*Corresponding author emails: darshana.bhosle@gmail.com & ash_arekar@rediffmail.com

ABSTRACT
In vivo and *in vitro* plant extracts of *Momordica dioica* Roxb. (Cucurbitaceae) were tested against a range of Gram positive and Gram negative bacteria. *In vitro* shoot cultures were obtained from seed explants of *M. dioica* on MS basal medium supplemented with 8.88 µM BA. *In vitro* callus culture was obtained from leaf explant of *M. dioica* on MS basal medium supplemented with 8.88 µM BA and 1.08 µM NAA. The Ethyl Acetate fractions of Methanolic extracts of *in vivo* and *in vitro* plant material of *M. dioica* showed significant antibacterial sensitive Activity.

KEY WORDS: *In vitro* shoot cultures, Callus Culture, *Momordica dioica*, Antibacterial activity.

INTRODUCTION

Momordica dioica (Roxb.) is medicinally and economically important plant. It is a seasonal plant with tuberous root. It is cultivated for its edible fruits, used as a vegetable. Alkaloids are the major components found in the plant. Besides, it also contains β- sitosterol, glycosides, α - spinasterol - octadecanoate, α - spinasterol - 3 - O - β - D - glucopyranoside, 3 - O - β - D - glucuronopyranosyl gypsogenin, 3 - O - β - D - glucopyranosyl gypsogenin. The root consists of 3 - β - O - benzoyl - 11 - oxo-ursolic acid, 3 - β - O - benzoyl - 6 - oxo-ursolic acid, Oleanolic acid, Gypsogenin, α-spinasterol, Hedragenin, Stearic acid [1,2]. The plant is reported to have antimicrobial [3], antimalerial [4], antitumour and anticancer [1] properties.
Flavonoids are the major components of many plant drugs which occur in arial part of the plants [5]. Flavonoid is a group of naturally occurring benzo-γ-pyrone derivatives that has been observed to possess several biological properties such as anti-inflammatory, antiviral, antibacterial, antifungal and insecticidal [6]. However, the bitter flavonoids and the other flavonoid compounds found in plants possess anti-infective properties [7, 8]. Ebi *et al*., [9] extracted flavonoids and tannins from Ethyl Acetate soluble fractions of Methanolic extract of *Uvaria chamae,* which showed significant antimicrobial activity. In the present paper the efforts are made to carry out the Antibacterial Activity of the plant using Ethyl Acetate extracts.

EXPERIMENTAL

Collection of Plant material

The tuberous roots of *Momordica dioica* were collected from Dr. Balasaheb Sawant Krishi Vidyapeeth, Dapoli, India. The plants are grown and maintained in experimental green house of KET's Scientific Research Center, Mulund, India. The fresh leaves are used for tissue culture experiments. Leaf material collected from the same plants was dried at 37°C for 48hrs and powdered material was used for extraction.

In vitro Plant Material

Leaf and seed explants were surface sterilized under aseptic conditions and inoculated on MS basal medium supplemented with the appropriate concentrations of auxins and cytokinines. MS basal medium supplemented with 8.88 µM BA and 1.08 µM NAA gives greenish friable callus from leaf explant after 20-25 days. MS basal medium with 8.88 µM BA gives multiple shoots from seed explants. The cultures were incubated at 22 ± 2^0C and 16/8 hrs light/dark photoperiod. After four successive subcultures the callus and shoots were used for the extraction.

Extraction of Plant Material

Extraction of plant material was carried out by the standard procedure described by Wagner [10] for flavonoid compounds. The Ethyl Acetate fraction was separated in separating funnel and was allowed to evaporate. Dry weight of the fraction was taken (Table 1) and the sample was redissolved in Ethyl Acetate. *In vitro* callus and multiple shoots were extracted using same method. For the assay, 10mgs of sample was dissolved in 1ml Ethyl Acetate.

TABLE 1: Weight of ethyl acetate fraction collected from *Momordica dioica*.

Extracts	Weight in gms	% Flavonoid content
Leaf exract of *M. dioica*	0.029	2.9
Callus extract of leaf of *M. dioica*	0.215	21.5
In vitro shoot extract of *M. dioica*	0.013	0.26

Collection and Cultivation of microorganisms

The fresh cultures of *Escherichia coli, Staphylococcus aureus, Salmonella paratyphi and Proteus mirabilis* were collected from microbiology laboratory of King Edward's Memorial Hospital, Mumbai, India. The cultures were maintained by sub culturing it on nutrient agar medium and incubated at 37 ^{0} C for 24 Hours.

Antibacterial Assay

An assay was carried out by Standard Disc Diffusion Method described by Bauer [11]. The cultures were incubated at 37°C for 24hrs. Inhibitory activity of extracts was measured in terms of Inhibition Zone (mm).

RESULTS AND DISCUSSIONS

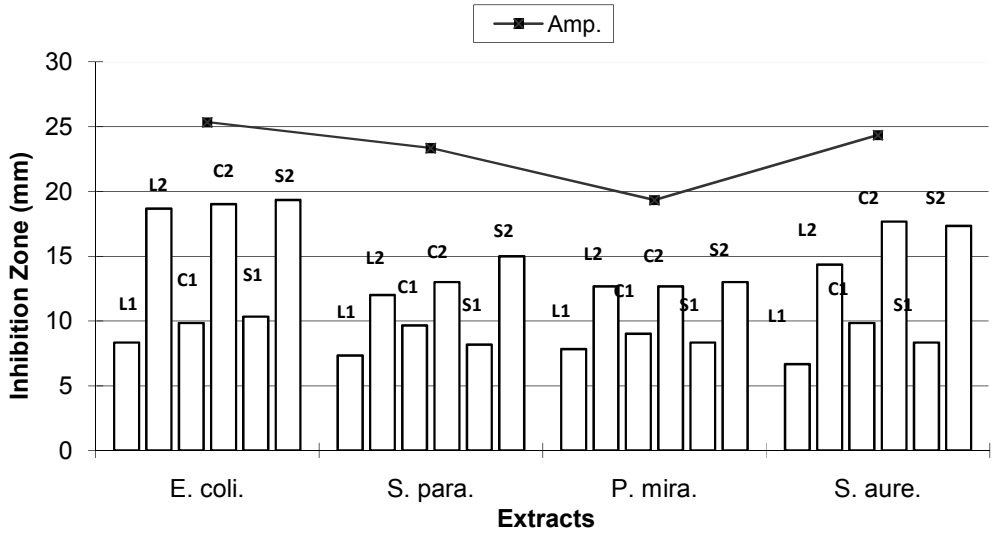
In present investigation we have obtained remarkable antibacterial activity of *In vivo* and *In vitro* Ethyl Acetate extract. The extracts with 200µg/ disc showed maximum inhibition. The same concentration (200µg/ disc) was more active against *E. coli* compared to *S. aureus, S. paratyphi,* and *P. mirabilis* bacteria. Ethyl Acetate extract of *In vitro* shoot culture (yield: 0.26%) showed maximum inhibition zone against *S. paratyphi,* and *P. mirabilis* while Ethyl Acetate extract of *In vitro* callus culture (yield: 21.5%) showed maximum inhibition zone against *S. aureus*. Table 2, Graph 1, shows that the Ethyl Acetate extract of *In vitro* plant material gave maximum inhibition than the *In vivo* plant material.

TABLE 2: Antibacterial activity of ethyl acetate fraction collected from *in vivo* and *in vitro* extracts of *Momordica dioica.*

Microorganism		Inhibition zone (mm)[a]						
		L_1	L_2	C_1	C_2	S_1	S_2	Amp
E. coli	G^-	8.33	18.66	9.83	19.0	10.33	19.33	35.33
S. paratyphi	G^-	7.33	12.00	9.66	13.00	8.16	15.00	33.33
P. mirabilis	G^-	7.83	12.66	9.00	12.66	8.33	13.00	29.33
S. aureus	G^+	6.66	14.33	9.83	17.66	8.33	17.33	34.33

[a] Values are the mean of three replicates. L_{1-2}: Leaf extract, C_{1-2}: Callus extract, S_{1-2}: Shoot extract, 1: 0.1mg, 2: 0.2mg.

GRAPH 1: Inhibition zone of *in vivo* and *in vitro* plant extracts against *E. coli, S. aureus, S. paratyphi, and P. mirabilis* with positive control.



CONCLUSION

In vivo and *in vitro* extracts of Ethyl Acetate were active against *E. coli, S. paratyphi P. mirabilis and S. aureus*. The obtained results show the broad spectrum of antibacterial activity which provides a support to more Eco friendly formulation. It may prove an alternative to conventional uses in a number of areas where antibacterial action is desirable. All the extracts are currently under chemical investigation in order to isolate the active constituents.

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