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Isolation of antimicrobially active compounds from the leaf of tribal edible plants of Tripura: *Momordica charantia* L. and *Paederia foetida* L.

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ABSTRACT

Components from methanolic leaf extracts of Momordica charantia L. and Paederia foetida L. were isolated. The physico-chemical studies and biological study was carried out and the structure of the most active component for both the extracts was elucidated using IR, NMR and MS spectral studies.

Keywords: *Momordica charantia* L., *Paederia foetida* L, Antibacterial, Antifungal, Structure.

INTRODUCTION

Momordica charantia (Family- Cucurbitaceae) is a herbaceous, tendril bearing vine which grows to 5m. The fruit is edible to the tribal people of Tripura, India, when harvested green and cooked. It tastes bitter. The fruit has twice the potassium of bananas and is also rich in Vitamin A and Vitamin C. It mainly contains glycosides, alkaloids, oils, glycoprotein [1]. In addition it also contains rosamarinic acid, rubixanthin, stigma-diol, stigmasterol [2]. The bitter fruit has demonstrated antidiabetic and hypoglycemic activity [3-7]. In addition it is known to have antioxidant and chemo protective property [8], antimicrobial activity [9], and cholesterol-lowering effects [10-12], anticancer and cytotoxic actions [13-15] and anti-ulcer actions [16, 17].

Paederia foetida L. (Family- Rubiaceae) is an aggressive competitive vine with a foetid smell growing high into the canopy of trees in a variety of habitats. It grows well in Eastern and Southern Asia, North America and South America [18]. Its leaves contain methyl mercaptan and aerial parts contain crystalline keto alcohol, paederolone, paederone and hetasitosterol [19]. It has antinociceptive activity [19], anti-inflammatory effects [20], and anti-diarrhoeal activity [21]. Traditionally the plant may also be used for the treatment of piles, inflammation of spleen [19], and also for toothache [19], and rheumatism [22].

MATERIALS AND METHODS

Plant materials (*Momordica charantia* L and *Paederia foetida* L) were collected from Agartala, Tripura (west), India, and authenticated by the Department of Pharmacognosy, RIPSAT, Agartala, Tripura, India and is extracted with methanol (A.R.) in Soxhlet Apparatus. Column Chromatography was performed by taking ethyl acetate as mobile phase and silica gel as stationary phase. ^{1}H and ^{13}C NMR spectras were recorded on BRUKER AMX 500 MHz NMR spectrophotometer using TMS as an internal standard. IR spectra were recorded by using JASCO 5300 series FTIR- spectrophotometer. Mass spectra were recorded on SHIMADZU 2010A series LC-MS system. The melting points were determined by using MELTING POINT APPARATUS, INDO, M-AB-92.

Table No.1-Characterization of extracts

Plant Parts of	Colour	pH	Density (g/cm^3)	Specific Gravity
Momordica charantia	Very deep green	6	0.795	0.7973362
Paederia foetida	Deep green	6.5	0.77	0.7722627

Density of water at 25.5 ◦C = 0.99707 g/cm^3

Table No.2 - R_f Values

Extracts	Solvent System	Spot Number	Distance traveled by components (cm)	Distance traveled by solvent (cm)	R_f Values
Momordica charantia	B	1	0.2	5	0.04
		2	1.9	5	0.38
		3	3.1	5	0.62
		4	4	5	0.8
		5	4.9	5	0.98
	BAW	1	0.9	5	0.169
		2	2	5	0.37
		3	3.2	5	0.602
		4	4.6	5	0.86
		5	5.2	5	0.97
	BWD	1	0.5	5	0.1
		2	1.9	5	0.39
		3	2.5	5	0.52
		4	3.6	5	0.75
		5	4.4	5	0.196
Paaederia foetida	B	1	0.4	5	0.07
		2	1	5	0.19
		3	1.6	5	0.3
		4	2.2	5	0.42
		5	2.7	5	0.51
	BAW	1	2.1	5	0.42
		2	3.1	5	0.63
		3	4.2	5	0.85
		4	4.8	5	0.97
	BWD	1	0.3	5	0.05
		2	1.2	5	0.22
		3	1.9	5	0.35
		4	2.8	5	0.51
		5	3.8	5	0.7

Butanol : Acetic Acid : Water (4 : 2 : 1) = BAW
Butanol : Water : Dioxan (4 : 2 : 1)= BWD
Benzene = B

Physico-chemical Studies

The extracts were studied for pH, density, specific gravity [23] and colour and the results were reported in Table No.1.

The pH of the extract was determined by using a pH meter (ECPC TUTOR, Eutech Instruments, pH tutor, Singapore, Sl. No. 242510), where the meter was calibrated by using buffer tablets of pH 4 , pH 7 and pH 9. Density bottle was used to determine the density of the extract at room temperature and the specific gravity was calculated accordingly.

Thin Layer Chromatography was performed using Silica Gel G as stationary phase and 3 different mobile phases : i) Butanol : Water : Dioxan = 4 : 2 : 1., ii) Butanol : Acetic Acid : Water =4 : 1 : 1 and iii) Benzene. The spots were visualized in an Iodine-Vapour chamber and marked accordingly. The R_f [24, 25] values were determined and results were given in Table No. 2.

Qualitative analytical [26] tests were also carried out and results were depicted in Table No. 3. All the chemicals and reagents used were of analytical grade.

Table No. 3 - Qualitative analytical Tests

Plant parts of	Constituents present
1. *Momordica charantia L.*	Alkaloids, Saponins
2. *Paederia foetida L.*	Alkaloids, Reducing sugar, Saponins, Fixed Oil, Amino Acid

Components present in the extract were separated by using a Column Chromatography taking Ethyl Acetate as mobile phase and Silica Gel for Column Chromatography as stationary phase. Total volume of extracts 500 ml were subjected for separation purpose. Separated parts were collected in individual beakers and solvents were allowed to evaporate at room temperature. The solid was collected and processed further. The colour of the components and volume obtained is given in Table No. 4.

Table No. 4 - Column Chromatography

Name of the plant	Components seperated	Colour	Volume (ml)	Weight (mg)
Momordica charantia	U_1	Deep green	100	30
	U_2	Green	85	23
	U_3	Greenish yellow	125	30
	U_4	Yellow	107	25
	U_5	Yellowish pink	45	10
	Absorbed volume		40	
Paederia foetida	G_1	Deep green	130	25
	G_2	Greenish yellow	245	38
	G_3	Light yellow	85	12
	Absorbed volume		40	

The melting points of the antimicrobially active components were determined and were uncorrected and were depicted in Table No.5.

Table No.5 - Melting point determination of active components

Name of the Plants	Components	Melting Points (◦C)
Momordica charantia	U_4	132
Paederia foetida	G_2	98

Table 6 – Antimicrobial Activities of Separated Fractions

Extracts of Plants/ Standard	Antibacterial activity[#] [Diameter of the inhibition zone (mm)]				AntifungalActivity[#] [Diameter of the inhibition zone (mm)]
	Bacillus pumillus	***Staphylococcus aureus***	***Escherichia Coli***	***Vibrio cholarae***	***Candida albicans***
***Momordica charantia* Leaves(U)**	19 (500)	11 (1450)	16 (750)	18 (550)	13 (1200)
U_1	10 (1500)	5 (>1500)	18 (575)	0 (>2000)	0
U_2	15 (850)	4 (>1500)	9 (>1500)	0 (>2000)	0
U_3	16 (700)	12 (1450)	6 (>1500)	14 (1000)	10 (>1500)
U_4	16 (800)	11 (1450)	7 (>1500)	13 (1250)	17 (600)
U_5	12 (400)	11 (1500)	7 (>1500)	14 (1000)	15 (900)
***Paederia foetida* Leaves(G)**	17 (650)	6 (600)	19 (550)	10 (1500)	12 (1400)
G_1	17 (650)	6 (1500)	19 (500)	10 (1500)	12 (1400)
G_2	0 (>2000)	13 (1200)	8 (>1500)	15 (900)	11 (1000)
G_3	18 (500)	8 (>1500)	18 (550)	15 (850)	13 (1250)
Tetracycline (Standard)	23 (30)	24 (250)	24 (27)	23 (30)	NA
Amphotericin B (Standard)	NA	NA	NA	NA	16 (50)

[#]*Concentration was 200 µg/mL.*
0: No Inhibition zone
NA: No Activity done

Biological Activity

The antimicrobial activity of all the extracts was determined by paper disc method [27] in nutrient agar (antibacterial activity) and Sabouraud dextrose agar (antifungal activity) medium. In this work, two gram positive bacteria - *Bacillus pumillus* (ATCC-7061), and *Staphylococcus aureus* (ATTC-25923); two gram negative bacteria - *Escherichia coli* (ATTC-25922), and *Vibrio cholarae* (recultured) were used to evaluate in-vitro antibacterial activity. They have also been assayed for antifungal activity against *Candida albicans* (recultured). The activity was determined in-vitro by measuring zone of inhibition in mm. All the fractions were tested in concentration of 200µg/ml and compared with standard drug Tetracycline and standard Amphotericin B. Minimum Inhibitory Concentration (MIC) [28], which is defined as the lowest concentration of inhibitor at which bacterial growth was not visually apparent, of all the extracts and the entire separated fraction were also determined by observing optical density at 600 nm by following serial dilution technique. Results are displayed in Table No. 6. Both the extracts were able to inhibit the growth of all the organisms. Separated components of *M.charantia* were not able to show more activity than crude extracts in *Bacillus pumillus*, but components **U_3, U_4, U_5** were showing at par activity with *Staphylococcus aureus.* Component **U_1** of *M. charantia* was showing more activity than the crude extract with *Escherichia coli*, but no component was able to show activity in comparison to the crude extract in *Vibrio cholarae.* More activity was recorded for **U_4** and **U_5** components of *M. charantia* than the crude extract in case of *Candida albicans.* It could be assumed that **U_4** component of *M. charantia* is having more activity than

the others. **G_2** component of *P. foetida* was able to show more activity than crude extract in case of *Bacillus pumillus.* Again it was more active against *Staphylococcus aureus* also. This component was also able to show more / at par activity than the crude extract in case of *Escherichia coli, Vibrio cholarae* and even *Candida albicans.*

RESULTS AND DISCUSSION

Plant materials (*Momordica .charantia* and *Paederia foetida*) were collected, shed-dried and pulverized with a mechanical grinder and then is extracted with methanol (A.R.) in Soxhlet Apparatus. The two extracts obtained were studied for their pH, density and specific gravity. All the results were reported in Table No. 1.

TLC was performed for each of the extracts *Momordica charantia* and *Paederia foetida* on Silica gel G coated plates, used as stationary phase. 3 mobile phases were used for each of the extracts: i) Butanol : Water : Dioxan = 4 : 2 : 1., ii) Butanol : Acetic Acid : Water =4 : 1 : 1 and iii) Benzene. The spots were visualized by Iodine-Vapour chamber and R_f [25, 26] values were determined. Results are displayed in Table No. 2.

The extracts were also subjected for qualitative chemical tests showing the results in Table No.3 using analytical grade chemicals, solvents and reagents. *Momordica charantia* L. contains alkaloids, saponins and *Paederia foetida* L. contains alkaloids, reducing sugar, saponins, fixed oil and amino acid.

Both the extracts were then Column chromatographed for separation of fractions. Five fractions of *Momordica charantia* (U_1 - U_5) and three fractions of *Paederia foetida* (G_1 – G_3) were obtained. The Column Chromatography was performed using Ethyl Acetate as the mobile phase and Silica Gel for Chromatography as stationary phase. The separated fractions were then collected. The colour and volume of all the separated fractions of the both of the plant extracts are reported in Table No. 4.

The melting points of both the active compounds were determined and were uncorrected and given in Table No. 5.

Antimicrobial activity

The extracts and all the separated fractions have been evaluated in-vitro for their antibacterial activities against *Bacillus pumillus* (ATCC-7061), *Staphylococcus aureus* (ATTC-25923), *Escherichia coli* (ATTC-25922), and *Vibrio cholarae* (recultured). They have also been assayed for antifungal activity against *Candida albicans* (recultured). All the compounds were used in a concentration of 200μg/ml and compared with standard drugs Tetracycline and Amphotericin B. Inhibition was recorded by measuring the diameter of the inhibition zone at the end of 24 h for bacteria and 72 h for fungi. All the experiments were carried out in triplicate. Based on the results of zone of inhibition, the minimum inhibitory concentration (MIC) of all the extracts and the entire separated fractions were also determined by observing optical density at 600 nm by following serial dilution technique. Results are displayed in Table No.6. U_4 component of *M. charantia* was found to have more activity than the others. **G_2** component of *P. foetida* was able to show more activity than the others.

Structure Elucidation

Compound U_4

The compound U_4 in its IR spectrum showed the presence of hydroxyl group, carbonyl group and unsaturation by exhibiting absorption bands at 3404cm^{-1},1718 cm^{-1} and 1640 cm^{-1} respectively.

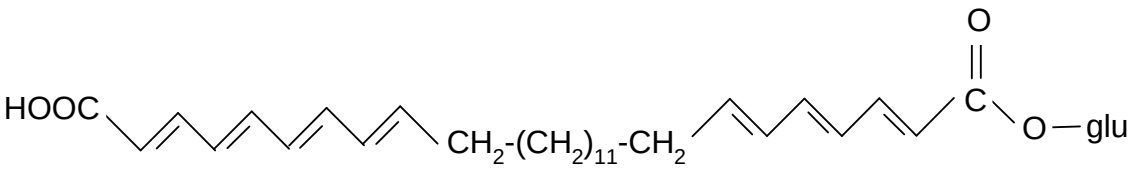
In its ^{1}H-NMR spectrum it exhibited signals in three different regions.

a. The signals at δ 0.85, 0.86, 1.16, 1.63, 1.73, 1.81, were due to long chain methylene groups and the signals at δ 2.25 and 2.35 were due to methylene groups adjacent to Carbonyl groups.
b. The broad signal at δ 3.33 was due to sugar moiety protons, probably glucose units.
c. The set of signals at δ 4.87, 5.10, 5.35, 5.37, 5.42 and 5.84 for anomeric protons and unsaturated protons.

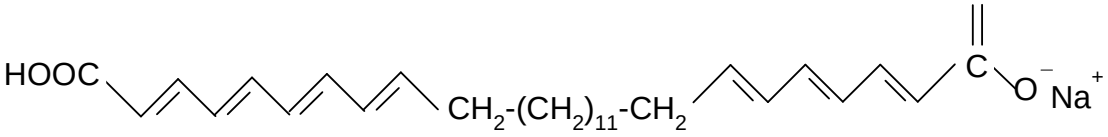
Consistent with the ^{1}H-NMR, the ^{13}C-NMR also indicated three different regions.

a. The signal at δ 104.95 was due to an anomeric carbon atom.
b. The fourteen signals 109.28, 113.83, 118.86, 124.56, 129.02, 130.118, 130.78, 131.33, 131.87, 132.30, 132.42, 133.84, 136.30 and 141.7 indicated the presence of 7 double bonds.
c. The signals at δ 18.078 and 185.34 were due to two carbonyl groups attached to unsaturated groups.
d. The methylene group signals (thirteen) δ 50.00, 47.76, 43.85, 36.84, 33.24, 30.33, 26.96, 24.08, 21.47, 20.38, 18.47, 17.08, and 14.1 appeared in the aliphatic region of the spectrum.
e. The signals at δ 83.50, 80.00, 75.84, 73.50, and 62.20 were due to a glucoside sugar moiety.

Considering the above facts the likely structure of the compound may be



Mass spectral fragmentation was recorded as under-



m/z 477

m/z 104

m/z 477 —— - COOH ——▶ m/z 432 —— - O ——▶ m/z 416

Compound G_2

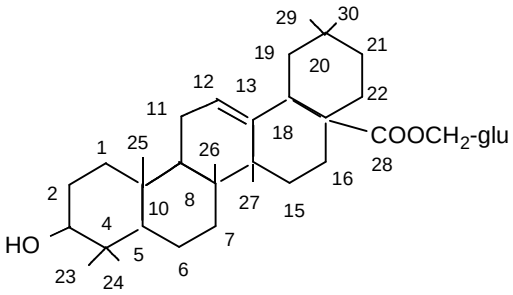
The IR spectrum of compound G_2 showed the absorption for hydroxyl groups (3400 cm^{-1}), ester carbonyl group (1741 cm^{-1}) and a double bond (1614 cm^{-1}) in the molecule.

The ^{1}H-NMR spectrum of the compound G_2 showed a multiplet at δ 2.39 (H-18), which was a characteristic signal for the H-18 of an ursane type nucleus, together with seven tertiary methyls at δ 0.67, 0.84, 0.87, 0.89, 0.99 and 1.25 (2 Me), the olefinic proton at δ 5.32 suggests a ursane type nucleus. Further it also exhibited signals within δ 3.13 to 3.60 indicating the presence of sugar moiety. It was supported by the bunch of signals at δ 67.90, 67.97, 71.17, 73.58 and 77.00. It also showed a strong signal at δ 2.50 for acyl protons.

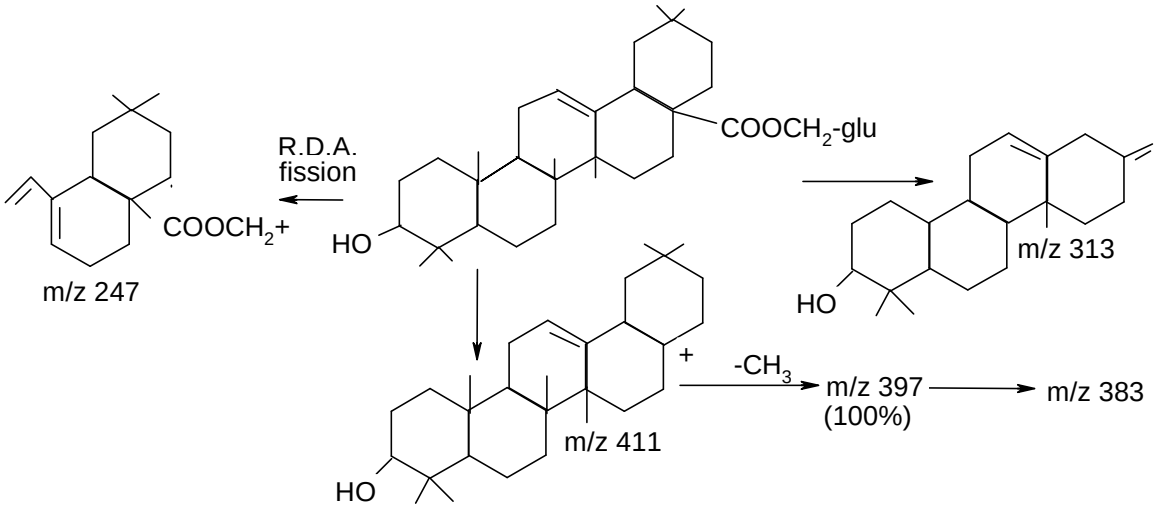
In the C^{13}-NMR spectrum the carbonyl signals being quaternary in nature (weak signals) it was very difficult to identify in the spectrum. The presence of a signal at δ 128.06 revealed that the double bond posses' only one hydrogen and the other side it was quaternary in nature. The aglycone part of carbon nucleus was reported in the following Table.

Position	δ_c	Position	δ_c	Position	δ_c
1	34.19	11	23.61	21	40.10
2	24.40	12	122.13	22	29.65
3	73.58	13	140.12	23	19.05
4	37.26	14	42.29	24	24.40
5	55.99	15	29.19	25	14.07
6	23.06	16	24.89	26	11.84
7	34.73	17	46.82	27	11.99
8	36.76	18	43.14	28	180.00
9	50.15	19	40.83	29	24.75
10	36.12	20	29.31	30	14.52

Considering the above data into account the tentative structure proposed as follows:



Mass spectral fragmentation of G_2 was recorded as under-



From the mass spectral fragmentations the ions at m/z 411, 397, 383 showed that the glucoside and the other groups are into the side chain and not in the pentacyclic part. Absence of the anomeric proton and anomeric carbon in the ^{1}H-NMR and ^{13}C-NMR spectrum indicated that it was C-glycoside. Glucose being a common sugar unit present in the phytochemical, the sugar moiety was considered as Glucose.

CONCLUSION

In conclusion, extracts of leaves of *Momordica charantia* and *Paederia foetida* were prepared. Their pH, density and specific gravity were determined. The extracts were Column chromatographed to obtain five fractions of *Momordica charantia* **($U_1 - U_5$)** and three fractions of *Paederia foetida* **(G_1-G_3)**.All the separated fractions and crude extracts were subjected to antimicrobial studies. **U_4** and **G_2** compounds were found to be most active and their structure was determined using IR, NMR, and MASS spectras. Further study is required to carry out the pharmacological activities of these antimicrobially potent compounds.

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