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EVALUATION OF PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITY OF *MOMORDICA COCHINCHINENESIS* AGAINST SOME PATHOGENIC MICROORGANISMS

Swathi Putta*, Eswar kumar Kilari[1], Nagendra Sastry[2]

*1 *A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India.*

[2] *Department of Biochemistry, GITAM University, Visakhapatnam, A.P, India.*

ABSTRACT

Phytochemical techniques played a significant role in searching raw materials and resources for pharmaceutical industry. Preliminary Phytochemical tests are helpful in finding and locating chemical constituents which are source of pharmacologically active principles. were carried out by employing standard methods for conducting Qualitative phytochemical analysis for studying the presence of active compounds like alkaloids, glycosides, phenols, saponins, tannins flavonoids, volatile oils, reducing sugars and steroids. Aqueous fruit extract of *Momordica cochinchinensis* (AFMC) showed presence of phenolic compounds, flavonoids, reducing sugars and glycosides except volatile oils, alkaloids, saponins, tannins and steroids. The antimicrobial activity of AFMC was studied by agar well diffusion method *in vitro*. The effect of antimicrobial potential was examined *Salmonella typhi, Vibrio cholerae, Shigella dysenteriae, Enterococcus faecalis.* The AFMC has showed consistently significant inhibitory activity on different bacterial species tested and found the significance of antimicrobial activity of *Momordica cochinchinensis.*

Keywords: *Momordica cochinchinensis,* Phytochemicals, Antimicrobial activity

INTRODUCTION:

In the recent years, antimicrobial resistance has become a major global problem[1]. Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants. Among the factors contributing to microbial resistance are indiscriminate use of antimicrobial agents by both healthcare professionals and patients, there is a need for production of substandard antimicrobials [2,3]. Nowadays herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimum side effects and relatively low cost[4]. Photochemicals are chemical compounds formed during the plants' normal metabolic processes[5].

Address for correspondence

Swathi Putta*

CSIR-SRF-extended
A.U.College of Pharmaceutical Sciences,
Andhra University,
Visakhapatnam. 530003
E-mail:swatidbm@gmail.com

These chemicals are often referred to as secondary metabolites of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids[6]. Many medicinal plants exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process [5]. There are reports on the curative potentials or abilities of medicinal plants and their products in the treatment of a wide range of infectious ailments such as urinary tract, gastrointestinal tract, respiratory tract and wound infections[7,8,9]. The potential value of such plant derived products prompted investigators to study new chemical constituents to improve the treatment of various diseases. Plants are the best source for the identification of new drug compounds.

The fruit of Momordica chochinchinenesis contains flavonoids like rutin, myricetin, luteolin, quercitin, apigenin and kaempiferol; carotenoids like α, β-carotene,zeaxanin, lycopene, lutein and phenolic compounds like gallic acid, vanillic acid, ferulic acid, caffeic acid, proto catechuic acid [10].

2. MARERIALS AND METHODS:

2.1 Plant Materials:

The aqueous fruit extract of *Momordica cochinchinensis* was obtained from Laila Implex, Vijayawada.

2.2 Phytochemical Analysis: The extracts were analyzed by the following procedures [11]. To test for the presence of the flavonoids, volatile oils, unsaturated sterols and or/ triterpenes, alkaloids, saponins, tannins, terpenoids, glycosides and reducing sugars

Flavonoids: 4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

Phenols: The Solvent plant extract was treated with few drops of neutral ferric chloride solution 5%, intense colour developed indicates the presence of phenols.

Volatile oils: 2ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is formed if volatile oils are present.

Saponins: Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Tannins: To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue color is observed for gallic tannins and green color indicates for catecholic tannins.

Anthroquinones: Borntreger's test was used for the detection of anthroquinones. 5 gm of plant extract was shaken with 10 ml of Benzene. This was filtered and 5.0 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of violet colour in the ammonical (lower) phase indicated the presence of free hydroxyl anthroquinones.

Reducing Sugars: To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

Glycosides: 25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

Alkaloids: 2ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.

Steriods: (Liebermann Burchard reaction: 200 mg plant extract in 10 ml chloroform, filtered), 2 ml filtrate + 2 ml acetic anhydride + conc. H_2SO_4. Blue green ring indicated the presence of steroids.

2.3 Test Microorganisms:

Salmonella typhi, Vibrio cholerae, Shigella dysenteriae and Enterococcus faecalis, are clinical isolates collected from King George Hospital, Visakhapatnam, India.

2.4 Anti microbial assay by agar well diffusion method:

The bacteria were grown in Muller-Hinton media (HiMedia Pvt. Ltd., Mumbai, India) at 37^0C while fungi were grown in Saboured Dextrose Agar media at 28^0C and maintained on nutrient agar slants at 40^0C and stored at -200 C. Inoculum of bacteria was prepared by growing pure isolate in nutrient broth at 37^0 C for overnight. The overnight broth bacterial cultures was sub-cultured in fresh nutrient broth and grown for 3hrs to obtain log phase culture. 21 days old grown fungi culture was scraped with sterile scalpel and dissolved in sterile saline solution to make different dilutions. The diluted suspension which has the absorbance of 0.600 at 450nm determined spectroscopically (Electronics India) then it was used as inoculums for fungi. The agar plates were prepared by pour plate method using 20ml of agar medium. The sterile agar medium is cooled to 45^0 C and mixed thoroughly with 1ml of growth culture of concerned test organism (1 x 108 cells) and then poured into the sterile petri dishes and allowed to solidify. Wells of 6 mm size were made with sterile cork borer and test extracts were added. The agar plates were incubated at for 4days at 28^0C for fungi wile 24hours at 37^0C for bacteria. The diameter of inhibition zones was measured in mm using HiMedia zone reader. Ciprofloxacin (Antibiotic) used as Standard while Solvent (DMSO) used for control [12].

2.5 Determination the Minimum Inhibitory Concentration by Broth Dilution Assay

The minimum inhibitory concentration (MIC) of the plant extract was determined using broth dilution assay. The medium containing different concentrations of plant extracts viz., 1mg -1µg per ml prepared by serial dilution (10-1 dilution). After inoculation of culture, the tubes were incubated for 72 hours at 28^0C for fungi while 24hours at 37^0 C for bacteria. The MIC of each sample was determined by measuring the optical density in the spectrophotometer (Electronics India) at 520nm and compared the result with those of the non-inoculated broth used as blank. Control was prepared using media and inoculums without plant extract [13, 14].

Table 1: Phytochemical analysis of aqueous fruit extract of *Momordica cochinchinensis*.

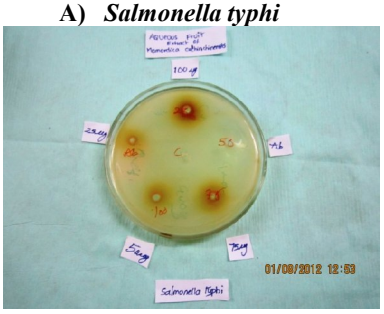
No	Phytochemicals	Aqueous fruit extract of *Momordica cochinchinensis*.
1	Flavonoids	+++
2	Volatile oils	---
3	Phenols	+++
4	Saponins	---
5	Tannins	---
6	Reducing sugars	+++
7	Glycosides	+++
8	Alkaloids	---
9	Steroids	---

Table 2: Anti microbial activity of AFMC extract on human pathogens.

S.no	Human pathogens	Causing Disease	Zone of inhibition of various diseases				
			25 µg	50 µg	75 µg	100 µg	Antibiotic(30 µg)
1	*Salmonella typhi* (G-ve)	Typhoid fever	5	6	7	8	10
2	*Shigella dysenteriae* (G-ve)	Dysentry	5	7	8	10	13
3	*Enterococcus faecalis* (G+ve)	Gastro intestinal infections	4	5	8	9	10
4	*Vibrio cholera* (G-ve)	Cholera	4	5	6	7	10

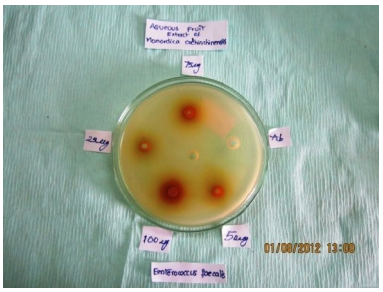
Fig 1: Antimicrobial activity of AFMC:

A) *Salmonella typhi*

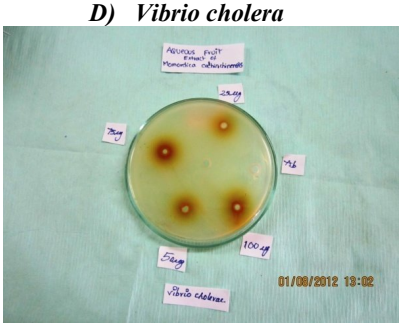


B) *Shigella dysenteriae*

C) *Enterococcus faecalis*



D) *Vibrio cholera*



3. RESULTS AND DISCUSSION:

3.1 Phytochemical Analysis:

Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes [15]. Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal science and are under investigation for Antibacterial, Antineoplastic and other Pharmaceutical functions [16]. Volatile oils are complex of compounds with strong odour. And known to have antiseptic, bactericidal, virucidal and fungicidal activities [17]. Phytochemical tests of the above extracts were performed through chemical reagents as described by Harbone 1998[18]. Nine chemical groups were screened for AFMC. The detailed investigations of phytochemicals in AFMC were shown in table 1. The AFMC shown to have positive results for presence of phenolic compounds, flavanoids, reducing sugars and glycosides. The alkaloids, volatile oils, saponins, tannins and steroids were found to be absent in aqueous fruit extract of *Momordica cochinchinensis.*

3.2 Antimicrobial activity on human pathogens:

Antimicrobial studies were carried out on human pathogenic bacteria and fungi. *Salmonella typhi, Vibrio cholerae, Shigella dysenteriae and Enterococcus faecalis*. The AFMC extract showed significant antibacterial activity. As shown in table 2, 8mm was the highest zone of inhibition against *Salmonella typhi*(G-ve) at 100 µg concentration of AFMC extract. 10mm was the highest zone of inhibition against *Shigella dysenteriae* (G-ve) at 100 µg concentration of AFMC extract 7mm was the highest zone of inhibition against *Vibrio cholera* (G-ve) at 100 µg concentration of AFMC extract 9mm was the highest zone of inhibition against *Enterococcus faecalis* (G+ve) at 100 µg concentration of AFMC extract. The AFMC extract showed comparable antibacterial activity with Ciprofloxacin (antibiotic) (Fig. 2). *S. aureus, E. faecalis* are Gram positive bacteria which were showed sensitive to extract compared to gram negative bacteria, this indicates that AFMC showed microbial growth static activity rather than microbicidal activity.

CONCLUSION:

In the present study was concluded that the presence of phytochemicals as phenolic compounds and flavanoids were present in AFMC. The strongest antibacterial activity of AFMC than the commercially available antibiotic Ciprofloxacin showed the maximum zone of inhibition against the selected species of microorganisms.

REFERENCES:

1. Raghunath D. Emerging antibiotic resistance in bacteria with special reference to India. *J Biosci.* 2008; 33 (4): 593-603.
2. Okeke IN, Lamikaure A, Edelman. Socioeconomic and Behavioural Factors Leading to Acquired Bacterial Resistance to Antibiotics in Developing Countries. *Emerg Infect Dis*. 1999; 5(1):1-9
3. Mourad AS, Metwally M, Nour EL, Deen A, Threlfall EJ, Rowe B, Mapes T, Hedstrom R, Bourgeois AL, Murphy JR. Multiple-drug resistant *Salmonella typhi*. *Clin and Infect Dis*. 1993; 17 (1):135-136.
4. Valiathan MS. Healing plants: *Current Sci.* 1998; 75: 1122- 1126
5. Okigbo R.N, Anuagasi C.L, Amadi J.E. Advances in selected medicinal and aromatic plants indigenous to Africa. *J. Med. Plants Res.*2009*;* 3(2): 86 – 95.
6. Okwu D.E 2004. Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. *J. Sustain. Agric Environ.* 6:30 – 34.
7. Navarro V, Villarreal ML, Rojas G, Lozoya X. Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. *J Ethnopharmacol*. 1996; 53 (3):143-147.
8. Eswar Kumar K, Swathi Putta, Nagendra sastry. Y, DSVGK Kaladhar, Govinda Rao. D. *In-vitro* antimicrobial and antioxidant activities of aqueous pericarp extract of *Punica granatum. Journal of Applied Pharmaceutical Science.*2013; 3 (08):107-112.
9. Govinda Rao D, Y. Nagendra Sastry, D.S.V.G.K. Kaladhar, K. Kamalakara Rao and K. Krishna Chaitanya. Antibacterial Activity Of Methanolic Seed Coat Extract Of *Borassus Flabellifer* L. *IJPSR*, 2011; 2(9): 2435-2438.
10. Jittawankubola, sirithonsiriamornpun. Phytovhemicals and antioxidant activity of different fruit fractions (peel, pulp, aril &seed) of thaigac(MomordicaCHochinchinensis). *Food Chemistry*. 2011;127: 1138-1145.
11. Talukdar AD, Choudhary MD, Chakraborty M and Dutta BK: Phytochemical screening and TLC profiling of plant extracts *Cyathea gigantea* (Wall. Ex. Hook.) Haltt. and *Cyathea brunoniana*. Wall.ex.Hook. (Cl.& Bak.). Assam University Journal of Science & Technology: *Biological and Environmental Sciences*. 2010; 5(1):70-74.
12. Govinda, R. D *et al*. Antibacterial activity of Methanolic Seed Coat Extract of *Borassus flabellifer* L. *Inter J Pharm. Sci. Res*. 2011; 2(9)
13. Andrews JM: Determination of minimum inhibitory concentrations. *J Antimicrob Chemother*. 2001; 48 (1): 5-16.
14. M07-A9 Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard – Ninth Edition 2012; 32(2):12-20.

15. Korkina, LG and Afanasev IB. Antioxidant and chelating properties of flavonoids. *Adv Pharmacol*, 1997; 38:151-63.
16. Yamunadevi M, Wesely EG, Johnson M. Phytochemical studies on the terpenoids of medicinally important plant *Aerva lanata* L. using HPTLC. *A. Pacific J. of Trop. Biomedicine.*2011, 220-225.
17. Bakkali F, Averback S, Idaomar M. Biological effects of essential oils. A review. *Food and chemical toxicology*, 2008; 446-475.
18. Harborne JB., Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. (3rd edition). Chapman and Hall Co., New York.1998; 1-302.

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